

The importance of haemoglobin to physical function

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I, James Otto confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Abstract

Haemoglobin (Hb) within erythrocytes establishes a fundamental link between oxygen (O_2) in ambient air and aerobic metabolism by transporting O_2 in the blood from lung to tissue. Historically, blood O_2 carriage has been quantified using the concentration of Hb in whole blood ([Hb]). Anaemia, defined as a fall in [Hb] below $130\text{ g}\cdot\text{l}^{-1}$ in males and $120\text{ g}\cdot\text{l}^{-1}$ in females may cause fatigue and impaired fitness. [Hb] is dependent on both plasma volume (PV) and total haemoglobin mass (tHb-mass). Thus, anaemia may result when tHb-mass falls but also when PV is expanded. Therefore, reliance on [Hb] may be misleading. tHb-mass may represent a better guide to O_2 carrying capacity and of aerobic fitness than [Hb]. The relationship between tHb-mass and [Hb] has, however, been studied little across disease states, nor has the relationship between tHb-mass and preoperative cardiopulmonary exercise testing (CPET) variables. I used the optimised carbon monoxide rebreathing (oCOR) method to determine tHb-mass, first seeking its test-retest reliability in healthy volunteers (HV). Typical error of repeat tHb-mass measurements was low in my hands, in keeping with other studies. Subsequently, the relationship between tHb-mass and [Hb] in HV and in different diseases was studied; inflammatory bowel disease (IBD), chronic liver disease (CLD), heart failure (HF) and those awaiting surgery. I found the relationship between tHb-mass and [Hb] varied considerably across disease states, with PV a key confounding variable affecting both [Hb] and the diagnosis of anaemia. Subsequently, in a separate cohort of surgical patients I determined the relationship between tHb-mass, [Hb] and physical fitness quantified by CPET. Unlike [Hb], I found tHb-mass to be an important variable associated with preoperative fitness. The oCOR method is feasible and well tolerated by patients and provides a more comprehensive assessment of haematological and volume status compared to relying on [Hb] and its future use is advocated.

List of abbreviations

ACI	Anaemia of chronic inflammation
AT	Exertional oxygen consumption at anaerobic threshold
ATP	Adenosine triphosphate
BMI	Body mass index
BNP	Brain natriuretic peptide
BV	Blood volume
BSA	Body surface area
CaO ₂	Arterial oxygen content
CaO ₂ -CvO ₂	Arterio-venous oxygen content difference
CLD	Chronic liver disease
CPET	Cardiopulmonary exercise testing
CO	Carbon monoxide
CO ₂	Carbon dioxide
⁵¹ Cr	Radiolabelled Chromium
CHF	Chronic heart failure
D _{O2}	Oxygen delivery
D _{O2crit}	Critical oxygen delivery
2,3-DPG	2,3-Diphosphoglycerate
eGFR	Estimated glomerular filtration rate
ETC	Electron transport chain
Hb	Haemoglobin
[Hb]	Haemoglobin concentration
95% CI	95% confidence interval
CR	Coefficient of repeatability
COHb	Carboxyhaemoglobin
%COHb	Percent carboxyhaemoglobin
Hct	Haematocrit
HV	Healthy volunteer
IBD	Inflammatory bowel disease
IDA	Iron deficiency anaemia
IQR	Interquartile range
LVEF	Left ventricular ejection fraction

MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
NHYA	New York Heart Association
O ₂	Oxygen
oCOR	Optimised carbon monoxide rebreathing
ODC	Oxyhaemoglobin dissociation curve
O ₂ ER	Oxygen extraction ratio
P ₅₀	Partial pressure of oxygen at which haemoglobin is 50% saturated
PaO ₂	Partial pressure of oxygen in arterial blood
PaCO ₂	Partial pressure of carbon dioxide in arterial blood
P _{ET} CO ₂	End-tidal partial pressure for carbon dioxide
P _{ET} O ₂	End-tidal partial pressure for oxygen
PV	Plasma volume
\dot{Q}	Cardiac output
RBC	Red blood cell
RCM	Red cell mass
RCV	Red cell volume
RDW	Red cell distribution width
RFH	Royal Free Hospital
rhEPO	Recombinant human erythropoietin
SaO ₂	Arterial haemoglobin saturation percentage
SD	Standard deviation
SV	Stroke volume
tHb-mass	Total haemoglobin mass
TE	Typical error
UCLH	University College London Hospital
$\dot{V}O_2$	Exertional oxygen consumption
$\dot{V}CO_2$	Carbon dioxide output
$\dot{V}O_{2max}$	Maximal oxygen consumption
$\dot{V}O_{2\ peak}$	Peak oxygen consumption
WHO	World Health Organisation
YLD	Years of life lived with disability

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Chapter 1

1 General introduction

Anaemia, meaning “without blood” in ancient Greek is a common haematologic disorder affecting both developing and developed countries and is quantified by a deficiency in the iron containing oxygen (O_2) carrier haemoglobin (Hb) stored within erythrocytes. The World Health Organisation (WHO) states that in the assessment of anaemia, “Hb concentration ([Hb]) is the most reliable indicator of anaemia at the population level” [1]. The WHO defines anaemia as an insufficient circulating [Hb] of $<130\text{ g}\cdot\text{l}^{-1}$ in men and $<120\text{ g}\cdot\text{l}^{-1}$ in women, with further adjustment made in pregnancy ($<110\text{ g}\cdot\text{l}^{-1}$) [2]. Anaemia can occur at all stages of the human lifespan but is more commonly an affliction in older age. From a worldwide perspective, anaemia is a global burden to human health with an estimated quarter of the world’s population affected. Specifically, anaemia affects 1.62 billion people (95% CI: 1.50–1.74 billion), which corresponds to 24.8% of the global population (95% CI: 22.9–26.7%) [2]. A systematic analysis of global anaemia burden spanning 1990 to 2010 revealed that anaemia accounted for 68.4 million years of life lived with disability (YLD) in 2010, equating to 8.8% of global total disability from all conditions [3]. Updated figures from the Global Burden of Disease, Injuries and Risk Factors Study in 2013 showed that the total number of anaemia cases had increased to 1.93 billion, which corresponded to an increased age-standardised prevalence of 27%, although YLD (61.5 million YLDs) decreased compared to figures from 2010 [4].

Iron deficiency is the single greatest underlying cause of anaemia (iron deficiency anaemia, IDA) at the global level in both sexes, accounting for 63% of the global total in 2013 [4]. IDA is also the leading cause of anaemia-attributable disability although the cause of anaemia does vary with geography, age and sex. Other leading causes of anaemia in both sexes are haemoglobinopathies (12% in females, 10% in males), gastrointestinal losses (5% in females and males), and malaria (5% in males) with gynecologic conditions (5%) unique to females and non-malarial infectious tropical diseases to males (4%) [3, 4]. In addition, many chronic (and especially inflammatory)

states also yield a 'functional iron deficiency' and blunted erythropoiesis, thereby resulting in 'the anaemia of chronic inflammation' (ACI) [5].

Anaemia is thus a clinically important haematological condition encountered in various clinical settings and across many different disease states from the surgical patient, where it affects around one third of cases preoperatively [6], to patients with inflammatory bowel disease (IBD) [7], liver disease (LD) [8] and chronic heart failure (CHF) [9]. Anaemia is associated with increased fatigue [10], impaired functional capacity [11, 12] and adverse surgical outcome [6, 13] as well as worsened prognosis in a variety of diseases from CHF [14], cancer [15] and chronic obstructive pulmonary disease (COPD) [16]. In the context of surgery, the reduced O₂ carrying capacity of blood in anaemic individuals with resulting tissue malperfusion may be one mechanism increasing susceptibility to adverse postoperative outcomes [17, 18].

For all these reasons, circulating haemoglobin concentrations are routinely measured in clinical practice. Once anaemia is identified, the cause of impaired haemoglobin synthesis or erythrocytosis, or of increased red cell destruction, is often sought and treatment (either of the underlying cause, or through the administration of packed donor red cells) initiated. However, it is now becoming clear that this approach may be somewhat simplistic. [Hb] may be an imperfect index of the blood's O₂ carrying capacity, being reduced not only when the absolute mass of circulating haemoglobin (tHb-mass) falls, but also by an increase in the volume of plasma (PV) in which the red blood cells (RBCs) are suspended. But a low [Hb] might also be found when haemoglobin synthesis and erythrocytosis are normal, and when tHb-mass is normal (or even high), if PV is disproportionately expanded. Therefore, both tHb-mass and PV can affect [Hb] independently of one another. Despite this, neither factor is routinely considered or measured in clinical practice. However, such assessment may be important if the drivers of an altered [Hb]- and the appropriate therapeutic response- are to be truly understood.

Total haemoglobin mass, being independent of PV may represent a better guide of Hb content and systemic O₂ carrying capacity given that each gram of Hb has the maximum capacity to bind 1.39 ml of O₂ and a trivial amount of O₂ is transported in plasma. Despite this, the relationship between tHb-mass and [Hb] has, however, been little studied in patient populations or across disease states and this is the first aim of my thesis to explore. Total haemoglobin mass can now be safely and reliably measured using the optimised carbon monoxide (CO) rebreathing (oCOR) method, refined by Schmidt and Prommer in 2005 [19]. The oCOR method has been widely used in sports medicine to measure tHb-mass and blood volume derivatives in elite athletes [20-29], following exposure to hypoxia (altitude training) [30-41] and in the context of blood doping [42-47], but has rarely been applied in the clinical setting [48-50]. If [Hb] is an imperfect index of tHb-mass and thus O₂ carrying capacity, then this raises important questions about the way in which we measure and quantify Hb and define anaemia in patients. For example, should tHb-mass be measured as an adjunct to or used instead of [Hb] as a means of assessing Hb in patients? Furthermore, do we need to readdress 'anaemia' as a concept and should we be defining volume excess and tHb-mass deficit separately instead?

Surgery, like exercise, places significant systemic hormonal and metabolic stress on the body. Whilst subject to some debate, O₂ supply at a rate inadequate to prevent skeletal muscle anaerobiosis may underpin the occurrence of the anaerobic threshold (AT) during exercise, although other mechanisms may include the recruitment of muscle fibres with differing metabolic features [51]. Regardless, the AT is an important submaximal marker of cardiorespiratory fitness [52] in health and disease. The maximal rate at which O₂ can be transported from the environment to the mitochondria and utilised to support oxidative phosphorylation is termed the maximal or peak oxygen uptake ($\dot{V}O_{2\max}$, $\dot{V}O_{2\text{ peak}}$). The classical view is that $\dot{V}O_{2\max}$ is constrained by the limits of the cardiovascular system [53, 54], although this is not without controversy [55]. Nonetheless, it is widely considered that O₂ delivery, and not O₂ extraction at the muscle which predominates in limiting $\dot{V}O_{2\max}$ in exercising humans [54], with cardiac output (\dot{Q}) and the O₂-carrying globular protein molecule Hb pivotal in this process [53].

Preoperative cardiopulmonary exercise testing (CPET) is considered the gold standard assessment of exercise capacity before surgery and is used to determine exertional $\dot{V}O_2$ at AT and $\dot{V}O_2$ peak, as measures of ability to meet increasing O_2 demands. The degree of surgical insult and the ability to meet the resulting additional postoperative O_2 demand appear to be fundamental determinants of surgical outcome: individuals in whom such ability is impaired, and thus with reduced $\dot{V}O_2$ peak and AT, are at greater risk of adverse surgical outcome [56-59]. The cause of uncoupling of O_2 supply and demand is multifactorial but likely an interaction between a patient's existing comorbidities (e.g. cardiac disease or respiratory disease), and other conditions that impact O_2 delivery &/or \dot{Q} . While \dot{Q} is well recognised as a key variable limiting exertional $\dot{V}O_2$ [53], the impact of anaemia and reduced concentration of oxygen-carrying Hb is less recognised, although others have shown associations between [Hb] and cardiorespiratory fitness [11] and enhanced exercise performance following acute changes in [Hb] [60].

I, and collaborators conducted a large, multicentre study of 1,777 patients awaiting elective surgery to try to address this by determining the association between preoperative [Hb] and cardiopulmonary exercise testing variables. This study (published during my PhD studies in 2013, see appendix G [11]) formed the foundations and background of this PhD and allowed a UCL Impact PhD Studentship to be successfully awarded. I found that to a weak extent anaemia (lower [Hb]) was independently associated with lower exertional $\dot{V}O_2$ at AT and $\dot{V}O_2$ peak, although this statistically significant finding may have been related to the large sample of patients studied, given that the correlation between [Hb] and $\dot{V}O_2$ at AT was only $r = 0.24$ and $r = 0.30$ between [Hb] and $\dot{V}O_2$ peak. In addition, only a very small amount of the variance in exertional $\dot{V}O_2$ at both AT and $\dot{V}O_2$ peak was explained by [Hb], suggesting that other factors may play a more important role in determining aerobic capacity such as maximal \dot{Q} (primarily maximum stroke volume), skeletal muscle characteristics (e.g. mitochondrial density and quality, muscle fibre typing, and microcirculatory function), pulmonary diffusing capacity and the O_2 carrying capacity of blood [54], the latter being highly dependent on tHb-mass.

To date, however, no studies have measured tHb-mass in the preoperative CPET patient awaiting major surgery. Therefore, the importance of tHb-mass as a determinant of CPET-derived physical fitness in patients prior to surgery remains unstudied and is the second aim of this thesis to explore. If tHb-mass is strongly related with exertional $\dot{V}O_2$, this provides a potential mechanism by which to enhance fitness for surgery and thus improve postoperative outcome. Overall thesis hypotheses and the aims of individual chapters are outlined below.

1.1. Hypotheses

- i) Total haemoglobin mass is poorly correlated with haemoglobin concentration, and
- ii) Unlike haemoglobin concentration, total haemoglobin mass is an important determinant of patient physical fitness associated with exertional oxygen consumption.

1.2. Overall aims and objectives of individual chapters

- i) Chapter 4:
 - a. To determine the test retest reliability of the optimised carbon monoxide rebreathing method in our laboratory, and
 - b. To quantify the repeatability of percent carboxyhaemoglobin (%COHb) measurements using the Cobas b 221 POC Hemoximeter (Roche Diagnostics, Rotkreuz, Switzerland).
- ii) Chapter 5
 - a. To determine the degree to which total haemoglobin mass is correlated with haemoglobin concentration in different patient populations and disease states, and
 - b. To assess the feasibility of using the optimised carbon monoxide rebreathing method in patients.

- iii) Chapter 6: To explore the relationship between both haemoglobin concentration and total haemoglobin mass and measures of patient physical fitness, as determined by cardiopulmonary exercise testing.

Chapter 2

2 Review of the Literature

This section outlines the key factors involved in and affecting O₂ transport, O₂ consumption and O₂ delivery before moving onto discuss how to quantify and define O₂ carrying capacity of blood. I then go on to discuss anaemia in varying clinical contexts before examining the relationships between markers of haemoglobin and objective measures of physical fitness.

2.1 Oxygen transport

O₂ must be transported effectively from the atmosphere to the tissues in order to maintain essential cellular metabolic pathways [61]. The transport of O₂ in the blood is achieved in main two ways; i) a small and trivial amount dissolved in the fluid portion of blood (plasma), and ii) the majority bound to the iron protein molecule Hb within red blood cells (RBCs) [51].

2.1.1 Dissolved oxygen

Under normal physiological conditions, a trivial amount of O₂ is dissolved in plasma, partly related to oxygen's relative insolubility in water, which keeps its concentration low in fluid. Specifically, the amount of O₂ dissolved in blood plasma is proportional to the partial pressure of oxygen (pO₂) (i.e. adheres to Henry's law [62]). Specifically, at a physiological pO₂ (being 100 mmHg in normal arterial blood), 0.3 ml O₂ per 100 ml of blood is dissolved [63]. This amount of O₂ is simply insufficient to sustain tissue O₂ requirements and an additional transport method is necessary to sustain life.

2.1.2 The haemoglobin molecule

Hb is an iron-containing globular protein pigment molecule carried within RBCs in the human body [64] and functions to carry almost all of the O₂ in the blood (98% of the total) [65]. Importantly, Hb establishes a fundamental connection between the O₂ available in ambient air and aerobic metabolism at the cellular level by transporting O₂ in the blood from the lungs to the tissues where O₂ is consumed and utilised to facilitate oxidative phosphorylation [66]. The structure of the HbA molecule can be seen below in Figure 2-1, comprising four polypeptide chains, two alpha (α) and two beta (β) chains forming two αβ-pairs [66]. Each polypeptide globin chain contains one porphyrin ring structure called heme, bound covalently with a central ferrous iron ion [67] which facilitates O₂ binding [68] in a reversible reaction to give oxyhaemoglobin (see Equation 2-1).

Equation 2-1. Oxygen binding with haemoglobin.

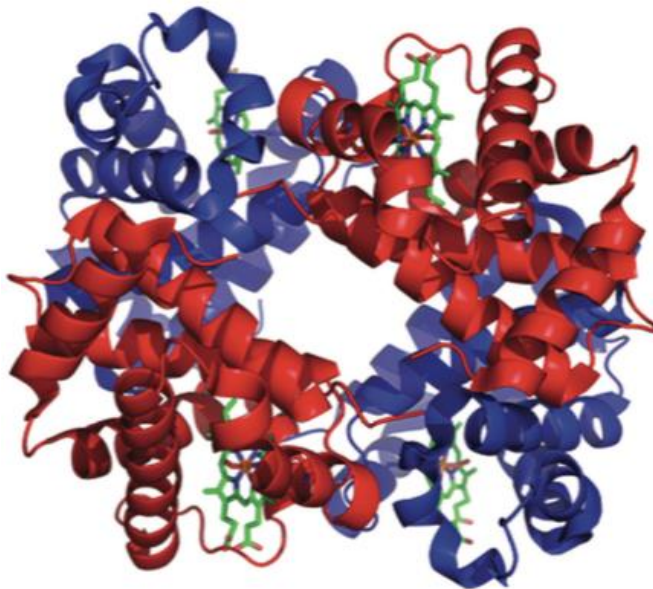
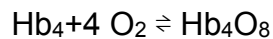


Figure 2-1. Structure of haemoglobin A showing in red, two α-chains and blue, two β-chains. In green are the four-iron containing heme groups.

The blood of a normal human adult contains at least 6 different forms of Hb molecule, largely with the same principle structure and function of which haemoglobin A (HbA) makes up approximately 95% of the total Hb concentration in a normal adult human [64]. When fully saturated with O₂, assuming a normal [Hb] level (e.g. 150 g·l⁻¹ in men) and a constant theoretical maximum O₂ binding capacity of Hb (Hüfner's constant of 1.39 ml·g⁻¹, see section 2.1.3 for discussion of Hüfner's constant), Hb carries nearly 20 ml of O₂ per dl of whole blood [64]. This reflects the maximum amount of O₂ that can be combined with Hb when all binding sites are occupied by O₂, this being the oxyhaemoglobin saturation.

Blood O₂ content is calculated from the sum of O₂ bound to Hb and O₂ dissolved in plasma and equates to the amount of O₂ in each 100 ml of blood. Oxygen content is calculated using the Equation 2-2 below (taken from [51]):

Equation 2-2. Calculation of oxygen content

$$Ca_{O_2} = (1.31 \times Hb \times Sa_{O_2} \times 0.01) + (0.0225 \times Pa_{O_2})$$

Where 1.31 is Hüfner's constant, Hb is the amount of Hb in grams per decilitre, Sa_{O₂} being the arterial Hb saturation percentage, 0.0225 the coefficient of solubility for O₂ at body temperature and Pa_{O₂} the partial pressure of O₂ in arterial blood (in kilopascals).

2.1.3 Hüfner's constant

Hüfner's constant refers to Hb O₂ carrying capacity and is defined as the maximum volume of O₂ that can combine with 1 gram of Hb [69]. The calculated/theoretical value of 1.39 ml of O₂ per gram commonly used is calculated from the molecular weight of Hb (64458 g), and that one molecule of Hb combines with four molecules of O₂ at a standard temperature and pressure

of 0°C and 760 mmHg, respectively [69]. However, there is debate over the O₂ combining capacity with Hb under physiological conditions [70]. As Gorelov [69] highlights, the volume of gas at 37 °C (i.e. body temperature) will be larger, and thus Hüfner's constant should be increased to reflect physiological conditions, so that the calculated value becomes 1.58 ml g⁻¹. However, given that other forms of Hb are present in the blood (e.g. carboxyhaemoglobin which contributes to 0.2 - 0.8% of total Hb in adults and methaemoglobin ~1% of total Hb), this reduces the capacity of Hb to bind with oxygen [71]. Therefore, the Hb O₂ binding capacity may be lower than when calculated. Taking this into consideration, McLellan and Walsh [70] suggest this could reduce the value of Hüfner's constant to 1.31 ml g⁻¹ (as used in Equation 2-2 above in the calculation of O₂ content). Despite this, it is still accepted that 1 gram of Hb has the maximum/theoretical capacity to bind 1.39 ml of O₂. Similarly, the maximum binding capacity of carbon monoxide (CO) to Hb is the same, i.e. one gram of Hb binds 1.39 ml of CO, as per Hüfner's constant.

2.1.4 P₅₀

The partial pressure of O₂ at which Hb is 50% saturated is called the P₅₀ [51] and is therefore a measure of Hb-O₂ affinity. A change in the P₅₀ value informs us as to whether the oxyhaemoglobin dissociation curve has shifted to the left or right [68], thus altering the affinity between O₂ and Hb.

2.1.5 Oxyhaemoglobin dissociation curve

The oxyhaemoglobin dissociation curve (ODC) shown in Figure 2-2 shows the saturation of Hb with O₂ at various pO₂ (mmHg) values. As displayed, this relationship is not linear but sigmoid in shape, which has a number of important physiological advantages as West highlights [63]:

- i) The upper right portion of the curve is flat meaning that if the pO₂ in alveolar gas falls, there will be limited impact on O₂ loading.
- ii) As RBCs take up O₂ along the pulmonary capillary, a large partial pressure gradient between alveolar gas and blood continues even

when most O_2 has been transferred, which hastens the diffusion process.

- iii) The lower left portion of the ODC results in peripheral tissues being able to withdraw/extract large amounts of O_2 for only a small drop in capillary O_2 , which assists the diffusion of O_2 into the tissues.

At sea level, alveolar pO_2 is 100 mmHg and Hb achieves 98% O_2 saturation. In other words, when partial pressure of O_2 is high, the affinity between O_2 and Hb ($Hb-O_2$) is very high (as occurs in the lung). In order to allow optimised O_2 delivery by Hb, a change in $Hb-O_2$ affinity is required that allows unloading of O_2 from the Hb molecule [72]. Several factors have been described that assist O_2 content loading in the pulmonary circulation and unloading of O_2 in the peripheral circulation, described below.

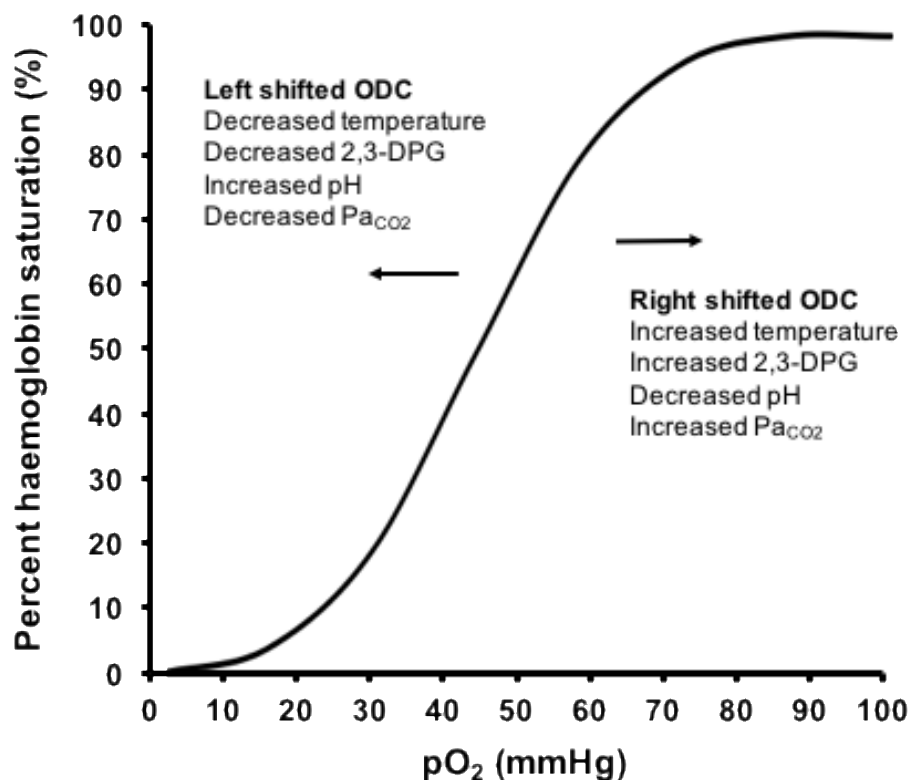


Figure 2-2. Oxygen dissociation curve. Adapted from [73].

As can be seen in Figure 2-2 there are various physiological and chemical factors that affect the position of the ODC and thus affect the P_{50} value and O_2 loading and unloading consequently. Specifically, a leftward shift in the ODC results in a reduction in the P_{50} value and increased $Hb-O_2$ affinity/binding and is

caused by factors outlined below, with a rightward shift in the ODC (resulting in an increase in the P_{50} value and reduction in Hb- O_2 affinity/binding) caused by the opposite [51, 74]:

- i) an increase in pH (reduction in hydrogen ions)
- ii) a reduction in temperature
- iii) a fall in arterial carbon dioxide content (P_{aCO_2})
- iv) a reduction in 2,3-diphosphoglycerate (being the organic phosphate found in RBCs that is produced as a consequence of glycolysis), which promotes Hb- O_2 release [51]

2.2 Oxygen delivery, consumption and extraction

The lungs, heart, vasculature and blood function to deliver a sufficient supply of O_2 , as well as substrate to the tissues to allow effective resynthesis of adenosine triphosphate (ATP) in the electron transport chain (ETC) [75]. Specifically, as shown in Equation 2-3 [75], O_2 delivery (D_{O_2}) is the product of cardiac output (\dot{Q}), arterial O_2 content (Ca_{O_2}) and Hb, where 1.39 is Hüfner's constant, being the amount of O_2 (in ml) carried per g of Hb at sea level.

Equation 2-3. Calculation of oxygen delivery

$$D_{O_2} = \dot{Q} \times Hb \times Sa_{O_2} \times 1.39 \text{ ml}$$

O_2 is the final step in this process by acting as the final electron acceptor in the ETC [76]. Without adequate O_2 flow from the blood to mitochondria, energy generating mechanisms within the mitochondria would come to a halt [77]. At sites with insufficient O_2 flow to mitochondria, anaerobic metabolism begins to predominate energy provision to complement ongoing aerobic ATP production [77]. This has particular relevance in the context of energy provision during exercise described below.

Approximately 90% of the body's oxygen consumption ($\dot{V}O_2$) is utilised in the ETC by the cytochrome-c-oxidase system as part of oxidative phosphorylation, which is the most efficient means of energy production in the body, yielding 37 ATP molecules from the complete oxidation of each glycosyl unit (see Equation 2-4) [78].

Equation 2-4. Aerobic phosphorylation



The quantity of $\dot{V}O_2$ has classically been expressed using the Fick equation, a product of heart rate and stroke volume, being cardiac output (\dot{Q}) and the arteriovenous oxygen content difference ($CaO_2 - CvO_2$), see Equation 2-5 [53].

Equation 2-5. Fick equation

$$\dot{V}O_2 = \dot{Q} \times CaO_2 - CvO_2$$

It is widely accepted or 'classically viewed' that the physiological parametric limits of the Fick equation determine the maximal rate at which O_2 can be transported from the environment to the mitochondria to support the oxidative phosphorylation of ATP and has been termed the maximal oxygen uptake or maximal oxygen consumption ($\dot{V}O_{2max}$) [53]. In health, although subject to debate, the finite rate of maximal oxygen consumption is determined by the ability of the cardiorespiratory system (heart, lungs, and blood) to transport O_2 to the muscles, not the ability of the muscle mitochondria to consume O_2 [54]. As such, $\dot{V}O_2$ dictates the amount of ATP that can be resynthesised aerobically.

Under normal physiological circumstances, $\dot{V}O_2$ remains unaffected by D_{O_2} over a wide range and thus remains supply independent [78]. This allows the body to initially manage a drop in D_{O_2} without disturbing aerobic respiration and $\dot{V}O_2$, which confers survival advantage and can be seen under stress conditions such as exercise or anaemia. This spare capacity is highlighted by normal resting values for D_{O_2} of $1000 \text{ ml}\cdot\text{min}^{-1}$ and $\dot{V}O_2$ of around $250 \text{ ml}\cdot\text{min}^{-1}$ [75].

This indicates that only 25% of the delivered O_2 is utilised by the tissues (based on a normal 70 kg adult undertaking normal daily activity) and represents the oxygen extraction ratio (O_2ER), being the ratio of $\dot{V}O_2$ to D_{O_2} [79]. As metabolic demand ($\dot{V}O_2$) increases or supply (D_{O_2}) is reduced, the O_2ER rises to maintain aerobic metabolism up until the maximum O_2ER is reached at 60-70% in most tissues. At this point, termed the critical D_{O_2} (D_{O_2crit}) being around $4 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in humans, $\dot{V}O_2$ becomes supply dependent [51]. Further increases in O_2 demand or impaired O_2 supply lead to tissue hypoxia [80], with subsequent anaerobic metabolism and lactate production [74].

As a consequence, anaerobic metabolism will supplement energy provision with resulting lactacidosis either when D_{O_2} falls below the D_{O_2crit} (as in heart failure, haemorrhage or hypoxaemia) or when O_2 demand increases beyond the ability of O_2 supply and tissue extraction (e.g. during heavy to severe exercise). At worst, a reduced D_{O_2} and impaired cellular utilisation of O_2 , which occurs across various circumstances such as severe anaemia can cause cellular hypoxia, cell death, organ failure and death [80]. The pathophysiological mechanisms underpinning the effects of anaemia are not yet fully understood and it may be the case that anaemia is associated with, rather than causative of unfavourable patient outcomes. Regardless it is important to outline the physiological responses to anaemia that lead to adaptive mechanisms to prioritise tissue D_{O_2} [81] [82]. These responses include [83]:

- i) an increase in \dot{Q} that is proportional to the extent of anaemia.
- ii) anaemia stimulates an increase in minute ventilation as well as nitric oxide (NO)-mediated improvement in ventilation-perfusion matching, leading to an increase in the partial pressure of O_2 in arterial blood and Hb oxygen saturation [84].
- iii) a reduction in systematic vascular resistance with preferential perfusion (through vasodilation) of vital organs (e.g. myocardium and brain). At the micro-circulatory level, capillary blood flow is increased and pre-capillary O_2 loss is reduced.
- iv) an increase in O_2 extraction.

- v) in chronic anaemia, 2,3-diphosphoglycerate (2,3-DPG) concentration increases, which promotes the offloading of O₂ to the tissues via a rightward shift in the ODC.
- vi) activation of hypoxic cellular mechanisms including neuronal nitric oxide synthase (nNOS) and hypoxic inducible factors (HIFs), which act to maintain O₂ homeostasis.

2.3 Haematological variables

This section initially pertains to definitions of haemoglobin content, blood volume and plasma volume. Thereafter I discuss measurement techniques to quantify blood volume and its component parts with particular focus on the optimised carbon monoxide rebreathing method to measure total haemoglobin mass.

2.3.1 Haematocrit and haemoglobin concentration

Haematocrit (Hct) represents the fraction of the blood volume comprised of red blood cells (normally expressed as a percentage) and is a measure of erythrocyte fraction/packed cell volume (PCV). Because Hct is based on whole blood it is dependent on PV [85] and therefore may not reveal accurate information pertaining to actual red cell volume (RCV), although it is often used as a surrogate marker of RCV. Therefore, for a 'true' assessment of red cell/haemoglobin mass, an independent measurement technique must be performed (see Section 2.3.5 for possible methods). Assuming a normal PV (which may not always be the case) in women, the normal reference range for Hct is 37-41% and 42-47% for males [86]. Therefore, Hct provides the volumetric relationship between red blood cells and plasma and importantly, Hct allows RCV or PV to be calculated once we have measured the quantity of the other [86].

The amount of O₂-carrying protein Hb in whole blood is termed the haemoglobin concentration ([Hb]) and is now expressed in grams of Hb per litre (g·l⁻¹), previously grams per decilitre (g·dl⁻¹). The change in units was bought in

by the Pathology Harmony Haematology Sub-group [87] for the terminology of the full blood count (FBC) to standardise units of measurement for both [Hb] and mean corpuscular haemoglobin concentration (MCHC) across all laboratories in the UK. There is some discrepancy in what normal reference ranges to use for [Hb], although typically for males between 130-180 g·l⁻¹ and females 120-160 g·l⁻¹ have been used [88]. Our local Haematology and Blood Transfusion User Handbook at University College Hospitals NHS Foundation Trust reports [Hb] reference ranges of between 115-155 g·l⁻¹ for females and 130-170 g·l⁻¹ for males [89]. Similar to Hct, the measured [Hb] value is also based on whole blood and is therefore highly dependent on PV.

2.3.2 Blood volume

BV is simply the sum of RCV and PV given that other whole blood components, specifically white blood cells and platelets comprise less than 0.1% of BV [90]. Like with many measurements in clinical and sports medicine, our interpretation of BV and its component parts is dependent on what is deemed normal. Traditionally, BV has been expressed relative to body weight (ml·kg⁻¹) with different ratios for males and females used although this adjustment may discriminate against obese and underweight individuals [90]. Alternative scaling techniques may be more appropriate such as relating blood volumes to lean body mass, although this is rarely carried out in practice. A wide range of average normal BV values have been reported (62-86 ml·kg⁻¹), which likely accommodates population variations in body weight to include underweight to overweight individuals [91]. The 1995 International Committee on Standardisation in Haematology [92] recommends a normal range of $\pm 25\%$ from the predicted norm while others have categorised BV based on the extent of deviation from predicted normal values [91].

2.3.3 Plasma volume

PV specifically relates to the intravascular portion of the extracellular fluid volume with the extravascular compartment characterised by fluid within the interstitial space [93]. Plasma is composed of around 92% water with additional

solutes such as plasma proteins, the most abundant of which being albumin [86]. Extracellular fluid volume is maintained within tight limits to maintain homeostasis in normal humans [94]. However, PV can be affected by body posture, exercise, hydration status and certain types of medication (e.g. diuretics) and PV regulation is underpinned by hormonal, neural, renal and cardiac mechanisms with the kidney playing a pivotal role [95] [90]. In this context, fluctuations in PV may cause haemoconcentration or haemodilution without any actual change in tHb-mass or RCV and therefore relying on [Hb] or Hct alone may not provide an accurate reflection of O₂ carrying capacity.

Indeed, data demonstrate that CHF leads to expanded PV without any reduction in RCV, so called 'pseudoanaemia' [96]. Similarly, others have reported that in patients with advanced CHF, haemodilution is common and is associated with worsened survival compared to patients with 'true' anaemia, suggesting that volume overload may be an important factor in anaemic CHF patients [97]. In contrast, PV has been shown to be contracted compared to controls in clinically stable patients with CHF receiving standard pharmacotherapy [98]. Fluctuations in PV have also been shown in patients with liver cirrhosis [99, 100], chronic severe anaemia [101], and patients during haemodialysis [102].

These studies highlight the importance of and justification for measuring tHb-mass or RCV and PV in addition to [Hb] and Hct and why [Hb] may be a poor guide of blood O₂ carrying capacity in several clinical circumstances. Despite this, there is a dearth of research assessing how well [Hb] reflects actual red cell mass across different patient populations and disease states.

2.3.4 Total haemoglobin mass

The total amount of Hb within the body, independent of compartmental fluid volumes is termed the total haemoglobin mass (tHb-mass) and is closely associated with the O₂ carrying capacity of blood given that each gram of Hb has the capacity to maximally transport 1.39 ml of O₂ and a trivial amount of O₂ is transported in plasma. tHb-mass, a component of red cell volume largely

determines arterial oxygen content (Ca_{O_2}), although Ca_{O_2} is also influenced by; i) [Hb], ii) the arterial oxygen saturation of Hb, iii) the binding capacity of Hb, iv) the actual arterial pO_2 and, v) the physical O_2 solubility of plasma [78].

2.3.5 Measurement of blood volume and its component parts

Validity, reliability and sensitivity are essential components of any measurement tool within clinical and sports medicine [103]. This section provides an overview of procedures used for the measurement of blood volume and its component parts (red cell volume and plasma volume), before moving on to discuss techniques to determine tHb-mass with particular focus on the optimised carbon monoxide rebreathing method.

The measurement of BV has been used in clinical medicine for the evaluation of anaemia, blood loss, polycythaemia, endocrine disorders and CHF [104, 105] as well as other applications in sports medicine such as among chronic exercisers [106], assessing adaptations to blood doping [107, 108] and altitude/hypoxic exposure [107, 109]. Measurement techniques to determine BV and its component parts have a long history, very much founded on the work by Haldane and Smith more than 110 years ago [110]. The underlying principle for the measurement of absolute blood volume is based on the indicator dilution technique [104]. It is important to state that the methods used to measure BV are indirect and are based on the observed dilution of a known amount of some appropriate substance or tracer entering the blood stream [90] that can be precisely and reliably measured. Equation 2-6 below underpins the methods that follow the indicator and dye-dilution principle. At its most simplistic level, if a known quantity of indicator (represented as A below) is administered into the system and its concentration (C) can be measured, then the desired, unknown volume (V) can be calculated.

Equation 2-6. Formula underpinning the indicator and dye-dilution principle

$$V = C/A$$

$$\text{Rearranged as } A = V \times C$$

Importantly, the other factor to consider is allowing sufficient time for complete and homogenous mixing of the indicator/tracer within the unknown volume. In this regard, the equation below is fundamental:

Equation 2-7. Time dependent indicator and dye-dilution principle

$$C_1 V_1 = C_2 V_2$$

Where:

C_1 = the concentration of the solution at baseline, prior to dilution

C_2 = final concentration of the solution, post dilution

V_1 = volume to be diluted

V_2 = final volume after dilution

Based on Equation 2-7, a known amount of tracer (A), contained in a known volume (V_1) with a known concentration (C_1), is administered in to a system whose volume (V_2) is unknown. A sample from V_2 is taken (after allowing sufficient mixing time in the circulation), thereafter the concentration of the tracer (C_2) can be sampled. Because the amount of tracer remains the same before and after mixing within the unknown volume, Equation 2-7 can be applied and rearranged (as shown in Equation 2-8 below) to calculate the unknown volume:

Equation 2-8. Rearranged formular for time dependent indicator and dye-dilution principle with a known amount of tracer

$$V_2 = C_1 V_1 / C_2 = A / C_2$$

Techniques to measure blood volume include the use of radioactively labelled RBCs with chromium (^{51}Cr) for the quantification of RCV, human serum albumin

labelled with iodine isotopes (^{131}I or ^{125}I) for the measurement of PV [111] and the Evans Blue method [112], which utilises a dilutional technique that measures PV by staining plasma proteins with dye [113, 114] and so does not involve the use of radioactive isotopes. The use of radioactive ^{51}Cr is widely considered the gold standard method for determining RCV in terms of accuracy [115, 116] as outlined by the International Committee for Standardisation in Haematology [116]. It is a common inaccuracy to use the terms red cell mass and red cell volume interchangeably as the ^{51}Cr method is a primary estimate of BV because counts in whole blood are compared with reference counts in the assay with subsequent measured red cell volume derived from BV using Hct [117, 118]. The use of ^{131}I or ^{125}I for the direct measurement of PV has been recommended [116], although its accuracy for the indirect measurement of RCV has been questioned [117]. In addition, using radioactive tracers in this manner are not without concerns over the health risks of using radioactivity as well as the time and costs involved with these techniques, which may to some extent limit their applicability and frequent use in the clinical setting given that they are restricted to the domain of the nuclear medicine laboratory.

Similar to the radioactive techniques discussed above, the measurement of tHb-mass is reliant on the dilution of a tracer such as carbon monoxide (CO). Inhaled CO is used because of its high binding affinity (some 220 times greater than O_2 [119]) with Hb that ensures it is absorbed and homogeneously distributed throughout the circulation, providing sufficient time is allowed for this to occur. CO was first proposed in this context as early as 1882 by Grehant and Quinquard [120] and subsequently by Haldane and Smith in 1900 [110]. Much more recently, modified techniques using CO rebreathing have been developed and refined by Thomsen and colleagues in 1991 [121], and Burge and Skinner in 1995 [122]. The Burge and Skinner method, which requires at least 10 minutes of rebreathing and venous blood sampling displays superior reliability to the Evan's blue dye technique and a high degree of validity when compared to the gold standard ^{51}Cr method [117, 122].

However, further refinement and optimisation of the CO rebreathing technique was undertaken by Schmidt and Prommer in 2005 [19]. Distinct and improved changes to the technique included a redesigning of the rebreathing spirometer

greatly enhancing the portability of the equipment and thus widening the settings in which the test can be performed, both in the laboratory and in the field. In addition, the use of a CO bolus to increase the rate of CO uptake into the circulation resulted in only 2 minutes of rebreathing being required to achieve complete CO distribution throughout the circulation, compared to 10 minutes of rebreathing in the Burge and Skinner method. This reduces the burden on the athlete or patient and lessens the time per test and therefore the optimised CO rebreathing method may be considered appropriate for more routine and regular use in the clinical and sporting arena.

Importantly, any measurement technique used to routinely assess tHb-mass must not be harmful or dangerous and should have negligible effects on physical performance and well-being, it should be inexpensive, user friendly and of low inconvenience for the patient or athlete. Previous techniques for measuring tHb-mass and blood volume compartments do not appear to have fully achieved these requirements. However, the 2-minute optimised carbon monoxide (CO) rebreathing (oCOR) method [19] provides an accurate, reliable and safe technique by which to quantify tHb-mass. This method is minimally invasive and has the lowest measurement error when compared to other blood volume techniques [117]. Despite this there is a dearth of research in the clinical setting that has utilised the oCOR technique, something this thesis will in part address through its use.

2.4 Definitions, prevalence and burden of disease

The following section provides a summary of definitions, prevalence and burden of the disease groups studied in this thesis. In particular, chronic heart failure (CHF), chronic liver disease (CLD), inflammatory bowel disease (IBD) and surgical patients (surgical) will be focused on. Finally, I will discuss anaemia in the context of specific disease states; CHF, CLD, IBD and surgery.

2.4.1 Chronic heart failure

The recently published European Society of Cardiology Task Force Guidelines for the diagnosis of acute and CHF [123] defines HF as “a clinical syndrome caused by cardiac abnormality of structural and/or functional origin that results in raised intracardiac pressures or impaired cardiac output at rest or under stress”. Therefore, the measurement of left ventricular ejection fraction (LVEF) either by echocardiogram or cardiac magnetic resonance imaging (cMRI) is the primary means of defining HF [123], although levels of B-type natriuretic (BNP) and/or N-terminal pro-B type natriuretic peptides (NT-proBNP) are used as an adjunct [124] for patients suspected of having HF and/or when HF is suspected in the absence left ventricular dysfunction [128]. In the non-acute setting, commonly used cut off values denoting the upper normal limits for BNP are 35 pg ml⁻¹ and 125 pg ml⁻¹ for NT-proBNP, with higher values applied in the acute setting [126]. In terms of LVEF (%), when < 40% the term HF with reduced ejection fraction (HFrEF) is used, when 40-49% HF with mid-range ejection fraction (HFmrEF) and when ≥ 50%, HF with preserved ejection fraction (HFpEF) is used [123].

HF is characterised by symptoms such as breathlessness, fatigue and ankle swelling that may occur alongside indications of elevated jugular venous pressure, peripheral oedema and pulmonary crackles [125]. The New York Heart Association (NYHA) class system, originally devised in 1923 [126] is the most widely used grading system to classify patients based on the severity of symptoms and the extent of functional impairment, see Table 2-1 on the following page.

Table 2-1. New York Heart Association Classification of heart failure symptoms.

Class	Symptoms
I	No limitations. Ordinary physical activity does not cause fatigue, breathlessness or palpitation. (Asymptomatic left ventricular dysfunction is included in this class)
II	Slight limitation of physical activity. Patients are comfortable at rest but ordinary levels of physical activity result in breathlessness, fatigue, palpitation or angina. Patients in this class are considered to have symptomatically “mild” heart failure.
III	Marked limitation of physical activity. Patients are comfortable at rest but less than ordinary physical activities lead to symptoms. Patients in this class are considered to have symptomatically “moderate” heart failure.
IV	Inability to carry out any physical activity without discomfort. Symptoms of heart failure are present even at rest and with any level of physical activity discomfort is increased. Patients in this class are considered to have symptomatically “severe” heart failure.

Amended from the National Institute for Health and Care Excellence (NICE) clinical guidelines for chronic heart failure [128].

HF occurs in the presence of several co morbidities that complicate and negatively affect patient outcomes [127]. Specifically, hypertension, coronary artery disease, atrial fibrillation, and chronic kidney disease demonstrate a direct causal relationship with HF while other common co-morbidities with varying clinical relevance include chronic obstructive pulmonary disease, diabetes, renal dysfunction, obesity and anaemia (with or without iron deficiency) [128]. It is outside the scope of this thesis to discuss all HF co-morbidities in detail but HF in the context of anaemia will be discussed in more detail below (see section 2.5.1).

HF is a significant public health burden and remains a rising global epidemic with prevalence of > 37 million individuals affected worldwide [129, 130]. In the United Kingdom around 900,000 people have HF [131, 132] with about 63,000 new cases each year [132], with HF burden greatest among the elderly [130]. These figures are likely to increase owing to the aging population and better management of heart disease [129]. Perhaps unsurprisingly, HF is associated with increased morbidity and mortality and therefore poor prognosis, although there has been a trend towards an improved HF prognosis over the past 10

year [125] as highlighted by a reduction in age-standardised death rates in England from 130.6 to 51.8 per 100, 000 people between 1981 to 2010 [133]. Despite this, as many as 30-40% of patients diagnosed with HF die within a year [134].

2.4.2 Chronic liver disease

Chronic liver disease (CLD) results from a progressive and chronic insult to liver parenchyma over an extended period of time leading to fibrosis and cirrhosis [135]. The strongest risk factor for liver cirrhosis is excessive alcohol consumption [136], with other leading causes of CLD being viral (hepatitis B and C), and non-alcoholic fatty liver disease (linked to obesity and diabetes). Other causes of CLD are genetic (haemochromatosis), autoimmune (autoimmune hepatitis), primary biliary cirrhosis and primary sclerosing cholangitis [137].

The global burden of LD is significant in terms of mortality and morbidity, with more than 1 million deaths and 31,027,000 disability adjusted life years (DALYs) due to liver cirrhosis alone [138]. In Europe, each year 170,000 deaths are related to liver cirrhosis (1.8% of all deaths) [137] and now in the UK CLD is the 3rd commonest cause of premature death [139]. Common complications of CLD include ascites, variceal bleeding, encephalopathy and hepatorenal syndrome [140].

2.4.3 Inflammatory bowel disease

Inflammatory bowel disease (IBD) encompasses two main relapsing disorders that are uncontrolled immune-mediated chronic inflammatory intestinal conditions, specifically Crohn's disease (CD) and Ulcerative colitis (UC) [141]. UC is non-transmural and commonly limited to the large bowel, whereas CD is transmural and can afflict the whole gastrointestinal tract [142], although UC and CD have overlapping clinical and pathological features [141]. On a global level there is significant variation in the prevalence of IBD taking into account geography, environment, immigration trends and ethnicity [143]. Updated in

August 2015, the World Gastroenterology Organisation Global Guidelines on IBD reported a European prevalence of 505 and 322 per 100,000 persons with UC and CD, respectively with an annual incidence of 24.3 and 12.7 per 100,000 person-years for UC and CD [141].

2.4.4 Surgery

The global volume of surgery is vast, with an estimated 234 million major surgical procedures undertaken every year worldwide [144]. In England between 2013-2014 there were 4.7 million surgical admissions, an increase of 27% from 2003-2004 with general surgery, trauma, orthopaedic and urology specialties showing the highest activity [145]. Data of over 46,000 unselected patients derived from 28 European countries included in the European Surgical Outcomes Study suggest that the crude mortality rate across all nations for patients undergoing inpatient non-cardiac surgery is 4%, with a median length of hospital stay of 3 (IQR 1-7) days, although wide variations existed across individual countries [146]. For example, in the UK crude in hospital mortality was 3.6% with values ranging from as low as 1.2% in Iceland to 21.5% in Latvia. In addition, data on over 4 million inpatient general surgical procedures in 94 NHS hospitals (spanning January 1999 and October 2000) obtained from the Intensive Care National Audit & Research Centre (ICNARC) and CHKS database supports the assumption that deaths are most common in older patients with pre-existing comorbidities. It is this 'high' risk population that, although comprising a relatively small percentage of surgical procedures (12.5%) accounts for a very large percentage of deaths (more than 80% in this cohort) [147].

This highlights the need for effective perioperative strategies to improve outcomes in high risk patients in particular, but also raises the importance of utilising effective and precise tools to assess perioperative risk in patients undergoing major surgery in general [148]. In addition, optimisation of patients preoperatively may offer an important opportunity to improve outcome. This may take the form of improving the management and treatment of pre-existing co morbidities (e.g. hypertension, diabetes, cardiovascular or respiratory

disease), optimising haemoglobin levels [149], enhancing cardiorespiratory fitness (prehabilitation [150, 151]) and through the adoption of enhanced recovery pathways [152].

2.5 Anaemia

The following section discusses the prevalence, causes and importance of anaemia in certain disease groups, specifically those patient groups studied in this thesis; chronic heart failure, chronic liver disease, inflammatory bowel disease and in patients awaiting surgery.

2.5.1 Anaemia and chronic heart failure

The prevalence of anaemia in CHF varies widely, in part related to a lack of consensus on a uniform definition, as well as comorbidities such as chronic kidney disease that may cause or worsen anaemia [153] and the clinical setting being studied (e.g. hospitalised patients, community/outpatient setting or patients included in randomised controlled trials) [154]. Regardless of the definition used or clinical setting, anaemia is common in patients with CHF, is related to the severity of disease and is associated with worsened symptoms and adverse outcomes [14] such as increased hospitalisations [155] and mortality [156]. A meta-analysis of 34 studies, comprising over 150,000 HF patients revealed 37% as anaemic (using criteria in the original articles to define anaemia) [157], and were almost twice as likely to die compared to non-anaemic patients even after adjustment for confounding factors such as age and renal dysfunction. A similar prevalence of 34% (this increased to 40% in patients aged > 70 years) was found in The Study of Anemia in a Heart Failure Population (STAMINA-HFP), a prospective study of over 1000 unselected outpatients with HF [158].

The most common cause of anaemia in CHF is iron deficiency, although many factors might independently contribute to the development of anaemia,

including but not exhaustively; chronic kidney disease (renal dysfunction) [159], chronic inflammation [160] also known as anaemia of chronic disease/inflammation (ACD/ACI), drug related (e.g. ACE inhibitors [161] and some beta blockers [162]), bone marrow dysfunction [163], and of particular interest to this thesis, haemodilution [97]. In addition, anaemia prevalence increases with age and is slightly higher in men than women in cohorts aged > 65 years [164].

2.5.2 Anaemia and chronic liver disease

Anaemia is a very prevalent haematological abnormality affecting patients with chronic liver diseases with a prevalence ranging from 50-75% being reported [165, 166]. There is a varied aetiology of anaemia in chronic liver disease [167], although a major cause is gastrointestinal haemorrhage (either acute or chronic) and hypersplenism linked to portal hypertension. In addition, a consequence of serious hepatocellular disease is a reduction in liver-producing clotting factors that would normally assist in lessening the effects of haemorrhage [168]. Given that the measured [Hb] is also dependent on plasma volume and that plasma volume is normally expanded/increased in patients with cirrhosis as a result of ascites and/or oedema, dilutional anaemia should also be considered as an aetiology of anaemia in chronic liver diseases [166, 169].

2.5.3 Anaemia and inflammatory bowel disease

A recent European meta-analysis of over 2000 patients with IBD reported an overall anaemia prevalence of 24%, which was higher in patients with CD (27% compared to UC (21%) [7]. An earlier systematic review found varying prevalence figures for anaemia in IBD with included studies reporting a prevalence ranging from 10 to 73% in CD and 9 to 67% in UC [170], perhaps related to different definitions of anaemia used and the clinical settings studied. For example, anaemia prevalence appears higher in hospitalised patients compared to the outpatients setting [171]. Overall these figures support the finding that anaemia represents a well known complication in IBD [172].

The most important causes of anaemia in IBD are iron deficiency, vitamin B12 deficiency and anaemia of chronic inflammation [172], which is closely linked to the regulation of iron homeostasis [173] and development of a functional iron deficiency [174, 175]. The predominant reasons for a high prevalence of iron deficiency in IBD (36 to 90% have been reported, depending on the definition used and cohort studied [171]) are chronic intestinal blood loss associated with the depletion of iron and iron stores [176] and impaired iron absorption caused by inflammation [177].

2.5.4 Preoperative anaemia

Approximately 25-30% of patients will present with anaemia prior to non-cardiac surgery. Data of 227,425 patients retrospectively extracted from the United States' National Surgery Quality Improvement Program (NSQIP) database demonstrates a prevalence of anaemia across all patients undergoing operation of 30.4%, equating to 69, 229 patients [13]. A similar prevalence (28.7%) was later confirmed by prospectively collected data from 28 European countries in patients undergoing non-cardiac and non-neurological surgery [6].

Furthermore, undertaking a meta-analysis of 897, 658 patients from fourteen studies, Fowler and colleagues [178] revealed a slightly higher prevalence of preoperative anaemia (40.3%) prior to non-cardiac surgery of various surgical specialities; vascular, orthopaedic, spinal, and upper gastrointestinal.

Importantly, existing co-morbidities (e.g. diabetes, heart failure, kidney disease and infection) and the condition or disease for which patients are undergoing surgery affects the reported prevalence of anaemia. For example, up to 75% of patients with Dukes Stage D colon cancer may be affected by anaemia [179].

These figures highlight that preoperative anaemia is a common affliction in the surgical patient and more prevalent than reported in the general population. Despite this, preoperative anaemia is often an incidental finding during pre-surgical workup and regularly an overlooked and under managed condition. This is in spite of considerable, albeit associative evidence of a relationship between preoperative anaemia and an increased risk of postoperative morbidity

and mortality following both non-cardiac [6, 13, 178, 180] and cardiac surgery [181-183].

2.6 Cardiopulmonary exercise testing (CPET)

Cardiopulmonary exercise testing, commonly abbreviated as CPX, CPEX or CPET is considered the gold standard assessment of cardiorespiratory fitness. CPET allows whole-body O₂ transport and utilisation to be estimated through the direct measurement of exertional O₂ consumption ($\dot{V}O_2$) [51], which is dependent on the integrated and dynamic function of the cardiovascular, pulmonary and musculoskeletal systems in response to increasing metabolic stress. CPET is used in sports medicine to assess the aerobic capacity of athletes and to monitor the effects of training and intervention longitudinally. In the clinical arena, CPET is routinely used in management of patients with heart failure [184, 185] as well as many other heart and lung diseases [186] such as cardiomyopathy [187], ischaemic heart disease [188, 189], chronic obstructive pulmonary disease [190] and pulmonary vascular disease [191, 192].

In the context of surgery, CPET is conducted for the purposes of preoperative risk assessment prior to major surgery to determine objective physical fitness and to assist in guiding appropriate level of postoperative care [193, 194]. The main CPET outcomes are the peak or maximal exertional oxygen consumption ($\dot{V}O_2$ peak or $\dot{V}O_{2max}$), being the highest or maximal rate at which O₂ can be utilised during exercise (typically averaged over the final 30 seconds of incremental exercise) and the exertional O₂ consumption at anaerobic threshold ($\dot{V}O_2$ at AT), defined as the point during incremental exercise when anaerobic metabolism begins to make a significant contribution to overall metabolism. Impairments in both $\dot{V}O_2$ peak and $\dot{V}O_2$ at AT (and thus an impaired physiological reserve above the resting state) are associated with an increased risk of postoperative morbidity and mortality [56, 58, 195] following major non-cardiac surgery. Conversely, it is deduced that a higher level of physical fitness (as quantified by an elevated $\dot{V}O_2$ peak and/or $\dot{V}O_2$ at AT during CPET) provides benefit (greater physiological reserve) to withstand the physiological insult of undergoing major surgery [51].

The original work by Older and colleagues almost two decades ago was the first to highlight an association between low functional capacity quantified by CPET and adverse patient outcomes following non-cardiopulmonary surgery [195]. Specifically, a reduced cardiorespiratory reserve, defined as an AT of less than $11 \text{ ml}\cdot\text{kg}^{-1}\text{min}^{-1}$ being associated with an increased risk of adverse postoperative outcome following major intra-cavity surgery [196]. Similarly, impaired $\dot{V}\text{O}_2$ peak has been shown to predict worse postoperative outcomes following major lung resection ($\dot{V}\text{O}_2$ peak $< 20 \text{ ml}\cdot\text{kg}^{-1}\text{min}^{-1}$ [197], $< 15 \text{ ml}\cdot\text{kg}^{-1}\text{min}^{-1}$ [198]) and bariatric surgery ($\dot{V}\text{O}_2$ peak $< 16 \text{ ml}\cdot\text{kg}^{-1}\text{min}^{-1}$) [199].

2.6.1 Preoperative cardiopulmonary exercise testing and major surgery

Major surgery can be defined as any intervention occurring in a hospital operating theatre involving the incision, excision, manipulation, or suturing of tissue, usually requiring regional or general anaesthesia or sedation [144]. The determinants of surgical outcome (morbidity and mortality) are related to an interaction between the health and fitness of patients, the number and severity of existing comorbidities present [200], as well as patient age and surgery-related factors (emergency or planned, mode, type and duration). In addition, the systemic inflammatory response caused by hormonal, immunological and metabolic mediators [201] is essential for effective tissue repair and healing after surgery. Effective O_2 delivery to the tissues during the hypermetabolic postoperative period is thought to be a fundamental determinant of surgical outcome [17, 202] with patients who are unable to raise O_2 delivery to meet the increased postoperative $\dot{V}\text{O}_2$ requirements more frequently developing complications [203, 204]. The cause of this uncoupling of O_2 supply and O_2 demand is multifactorial but may be predominantly linked to the interaction between a patient's existing comorbidities (e.g. cardiac disease, respiratory disease, or indeed any condition that impairs O_2 delivery &/or cardiac output) and the degree of surgical insult [205].

It is acknowledged that although the $\dot{V}O_2$ response from an exercise test is not directly comparable to that in a patient postoperatively, common with exercise, $\dot{V}O_2$ postoperatively following major surgery is high [206]. For example preoperative resting $\dot{V}O_2$ has been shown to increase from $110 \text{ ml}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$ to approximately $170 \text{ ml}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$ [207, 208] indicating a greater postoperative O_2 requirement and demand. In this context, tHb-mass may be important to surgical outcome due to its role in determining O_2 delivery. This may be related to the close linear relationship that exists between tHb-mass, BV, \dot{Q} and aerobic capacity [209]. For example, a high BV is a prerequisite for a high tHb-mass, which in turn impacts upon \dot{Q} by elevating venous return and cardiac filling pressures [210, 211]. Because tHb-mass in combination with PV also governs [Hb] and therefore O_2 carrying capacity, the effects of tHb-mass on determining O_2 delivery are twofold. Given the association between markers of cardiorespiratory fitness ($\dot{V}O_2$ peak and AT) and surgical outcome, it would seem intuitive that a high tHb-mass may confer a survival advantage in the perioperative setting. If this is the case, then strategies aimed at elevating tHb-mass may improve outcome (morbidity and mortality) following surgery, but this remains to be confirmed in appropriately powered studies. Given that anaemia is associated with an increased risk of adverse surgical outcome, it would be surprising if this relationship were not maintained for tHb-mass.

2.7 Haemoglobin and physical function

The following section reviews the effects of manipulating [Hb] on exercise capacity before discussing the association between changes in total haemoglobin mass and physical fitness.

The manipulation of Hb has historically been achieved through the transfusion of packed RBCs or erythropoietin stimulating agents such as recombinant human erythropoietin (rhEPO). rhEPO artificially stimulates erythropoiesis in addition to that which occurs naturally as a result of the glycoprotein hormone, erythropoietin (EPO) produced in the kidney [212]. Both methods, rhEPO and blood transfusion ultimately increase the numbers of circulating erythrocytes

and therefore the amount of O_2 carried in whole blood. This knowledge forms the physiological basis for the systematic and illegal practice of blood doping undertaken by an alarming number endurance athletes and coaches in the past. The assertion is that elevated $[Hb]$ increases arterial O_2 content and systemic O_2 transport (increasing D_{O_2} relative to $\dot{V}O_2$) with the additional O_2 available to the working muscles resulting in an increased $\dot{V}O_{2max}$ and athletic performance (e.g. faster running time, higher power output during cycling, etc) [213]. These same mechanisms (potential for increased D_{O_2} and increased physiological reserve) may also play an important role in the perioperative period.

2.7.1 Reductions in haemoglobin concentration and associations with exercise capacity

A consistent finding in the following literature is that a reduction in $[Hb]$ has a discernible effect on $\dot{V}O_{2max}$ and to a greater extent on exercise endurance that is proportionate to the reduction in O_2 carrying capacity. Ekblom and colleagues [214] demonstrated in four participants a 13% reduction in $[Hb]$ reduced $\dot{V}O_{2max}$ (measured during maximal treadmill running) by 10% (from 4.54 to 4.09 $l \cdot min^{-1}$), although a greater detriment was seen in submaximal cycling endurance time, which decreased by 30% from 5.77 to 4.04 minutes, following a single venesection of 800 ml of blood. In the same study an extra 4 participants underwent serial venesections of 400, 800, and 1200 ml of whole blood (4 days in between each) resulting in a fall in $[Hb]$ of 10, 15, and 18%, respectively. These reductions were manifested by a similar impairment in $\dot{V}O_{2max}$ (6, 10, and 16% reduction) and submaximal cycling endurance times (13, 21, and 30% reduction).

Similarly, Ekblom and colleagues [215] aimed to investigate the effect of different levels of arterial O_2 content on central circulation during both submaximal cycle ergometry and maximal treadmill exercise. After venesection of 800 ml of whole blood, Ca_{O_2} was reduced by 10% and $\dot{V}O_{2max}$ decreased from 4.27 to 4.03 $l \cdot min^{-1}$ (a 6% reduction). Ca_{O_2} was reduced post venesection during both submaximal cycling (from 182 to 166 $ml \cdot l^{-1}$) and maximal running

performance (from 199 to 179 ml·l⁻¹) and this was related to the change in [Hb] given that SaO₂ remained unchanged. Additionally, while exercising at higher submaximal workloads during cycle ergometry, blood lactate concentrations were lower in the “normal” state (5.9 mM) compared to the post venesection state (7.6 mM), which was inversely related to CaO₂ and suggests a reduced anaerobic energy contribution in a situation where O₂ availability is increased. This would suggest that one consequence of anaemia may be a greater lactate production during exercise [216].

Other authors to have reported similar findings include Balke and colleagues [217] who showed a 9% decrease in $\dot{V}O_{2\max}$ 1 hour after a 500 ml blood donation, Woodson and colleagues [218] who showed $\dot{V}O_{2\max}$ decreased by 16% after a 34% reduction in [Hb], and to a lesser extent Kanstrup and Ekblom [219] who demonstrated a 9% reduction in $\dot{V}O_{2\max}$ and 40% lower endurance time at the intensity eliciting $\dot{V}O_{2\max}$ after removing 900 ml of whole blood (resulting in an 11% reduction in [Hb]). Finally, Rowell and colleagues [220] showed a modest 4% decrease in $\dot{V}O_{2\max}$ following a 14% decrease in [Hb] after repeated venesections equating to 700-1000 ml over a 5 day period. The smaller detriment in observed $\dot{V}O_{2\max}$ (4%) by Rowell and colleagues may be explained by the use of the Astrand and Rhymin nomogram [221] as this has been shown to underestimate $\dot{V}O_{2\max}$ [220].

$\dot{V}O_{2\max}$ is the primary fitness outcome variable used in the majority of studies in this area, principally as it is the gold standard assessment of cardiorespiratory fitness and is largely considered to be constrained by the limits of the cardiovascular system. However, $\dot{V}O_{2\max}$ does not reflect the submaximal step-change metabolic demands encountered in every-day life such as when running for a bus or walking up stairs. Less is known about the impact of changes in [Hb] on submaximal markers of cardiorespiratory fitness, which the following section explores.

Burnley and colleagues [222] investigated the influence of blood donation on $\dot{V}O_2$ uptake kinetics, $\dot{V}O_2$ peak and time to exhaustion during severe-intensity cycling. The donation of approximately 450 ml of whole blood caused around a

5% decline in [Hb], from 15.4 to 14.7 g dl⁻¹ and a reduction in haematocrit from 44 to 41%. This corresponded to a 54 second (14%) reduction in the time to exhaustion and around a 4% decline in measured $\dot{V}O_2$ peak. Interestingly, the fall in [Hb] following blood donation was not related to the change in $\dot{V}O_2$ peak but was associated with the change in time to exhaustion ($r = 0.57$). In contrast to previous studies, no differences were found in blood lactate values at the end of exercise after blood donation [218, 219]. In addition, blood donation had no effect on the speed of $\dot{V}O_2$ on-kinetics, a finding in keeping with previous investigations in this area [223]. This may suggest that the relatively small reduction in O₂ delivery post blood withdrawal in this study was insufficient to affect $\dot{V}O_2$ kinetics or that adjustments in \dot{Q} (or its distribution to tissues) was sufficient to preserve muscle O₂ delivery. Nonetheless, this study confirms the importance of blood O₂-carrying capacity as a determinant of $\dot{V}O_2$ peak and to a greater extent exercise endurance, the latter perhaps being a more valid marker of functional status than $\dot{V}O_2$ peak when relating to activities of daily living, which are in general not performed at a maximal level of physical exertion.

Fritsch and colleagues [224] reported CPET data in 16 young healthy participants performing cycle ergometry before and two days post a single blood donation of 450 ml with resultant [Hb] falling from 14.5 to 13.0 g·dl⁻¹. The AT was reduced two days after blood donation when expressed as a percentage of $\dot{V}O_{2max}$ (68.5 vs 52 %) and in absolute $\dot{V}O_2$ at the AT. Similarly, data from Japan [225] suggest that the AT is lower in patients with iron deficiency anaemia compared to non-athletic controls (AT 15.9 ± 3.3 versus 21.3 ± 1.3 ml kg⁻¹ min⁻¹, $p < 0.01$) and is susceptible to change following iron supplementation when [Hb] is increased ([Hb] 9.0 ± 1.8 to 12.1 ± 0.8 g·dL⁻¹), AT (20.9 ± 6.3 to 25.0 ± 8.0 ml kg⁻¹ min⁻¹, $p < 0.001$).

A reduction in [Hb] and thus in O₂ carriage may impair exercise capacity in several ways. Firstly, a lower arterial O₂ content will reduce O₂ availability to the skeletal muscle for the same muscle blood flow [222]. Secondly, muscle O₂ diffusing capacity is lower when Hb is reduced, in part related to changes in the spacing between capillaries of erythrocytes and/or slower O₂ dissociation/unloading of O₂ from Hb [226]. Thirdly, a reduction in blood volume

as a result of acute blood withdrawal may impact upon aerobic capacity as changes in BV affect cardiac function, in particular ventricular preload via the Frank-Starling mechanism and thus influence the dispatch of O_2 per beat by altering SV and \dot{Q} (both being important determinants of $\dot{V}O_2$ as described in the Fick equation) [227, 228].

Similar mechanisms may underpin the reduced AT observed when [Hb] falls but this is a much-debated and controversial concept [229, 230]. As previously alluded to, the AT represents the highest $\dot{V}O_2$ before the metabolic demands of tissues (mitochondria) outstrip O_2 supply, and aerobic ATP resynthesis is supplemented by anaerobic metabolism leading to increased lactate production relative to the rate of glycolysis [231]. The AT is therefore an important marker of cardiorespiratory fitness as it provides an assessment of the ability of the cardiovascular system to supply O_2 at a rate adequate to prevent muscle anaerobiosis [52]. If this is the case, then a reduced capacity to supply O_2 to actively respiring tissues caused in part by low [Hb] or cardiovascular disease has the potential to reduce the AT.

2.7.2 Increases in haemoglobin concentration and changes in exercise capacity

Most published research studies discussed thus far have shown an association between a reduction in [Hb], impaired maximal exercise capacity and submaximal endurance. Is the converse true when [Hb] is artificially elevated? The following section explores this question.

Underpinned by the early work of Pace and colleagues in 1947 [232], a number of authors have reported data clearly showing that when [Hb] is elevated by the infusion of packed RBCs there is a reciprocal increase in $\dot{V}O_{2max}$ [214, 215, 233-239], see Figure 2-3 below. Where studies failed to find such a relationship [240] between an elevated [Hb] and physical fitness this may in part be explained by the quantity of blood reinfused being insufficient, or there being inadequate time for the body to adapt to its normal [Hb] after venesection [216]. When these factors are appropriately controlled for, elevating [Hb] increases

$\dot{V}O_{2\max}$ and endurance performance [233]. $\dot{V}O_{2\max}$ &/or exercise endurance has also been shown to increase in circumstances where [Hb] has been elevated by administering rhEPO in healthy individuals [241, 242], athletes [243], haemodialysis patients [244, 245] and patients with HF [246, 247] as well as following iron supplementation in young trained females [248].

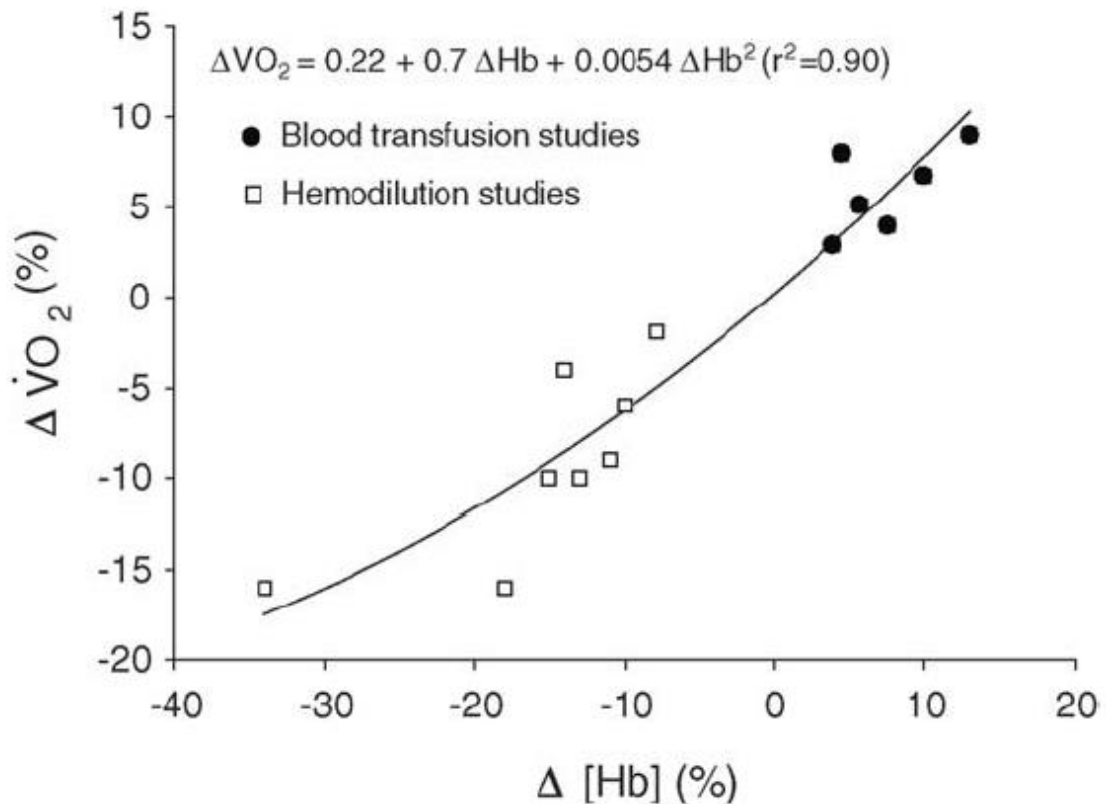


Figure 2-3. Relationship between the % change in haemoglobin concentration ([Hb]) and % change in $\dot{V}O_{2\max}$. Each data point represents the mean of each study using data obtained during the first 48 h after haemoglobin manipulation. Figure taken with permission from Calbet and colleagues [249] using data from 9 individual studies [214, 215, 217, 220, 234-236, 250].

In the clinical setting, the effects of blood transfusion on objectively measured cardiorespiratory fitness in adults is less well described, although the beneficial effects of blood transfusion on improving [Hb] and exercise capacity has been shown in children [251, 252] and young adults [253] with thalassemia. In a small study of anaemic adult patients with stable haematological conditions, Wright and colleagues [254] aimed to address this by studying the effects of blood transfusion on exercise capacity measured objectively with CPET

performed prior to and 3-5 days post transfusion of allogeneic packed red blood cells. Included patients were those aged over 18 years requiring transfusion for chronic anaemia as part of their routine hospital care. In eighteen patients, the mean \pm SD AT increased from 10.4 ± 2.4 to 11.6 ± 2.5 ml kg⁻¹ min⁻¹ following transfusion of a median (range) of 3 (1-4) units of packed red cells (mean \pm SD increase in [Hb] from 83 ± 12 to 112 ± 14 g.l⁻¹ after transfusion). However, in 5 patients the AT failed to increase following transfusion, perhaps related to a smaller increase in [Hb] in this group compared to patients in whom AT did significantly improve. Improvements in $\dot{V}O_2$ peak [(mean difference post transfusion (95% CI) of 1.5 (0.3-2.7) ml kg⁻¹ min⁻¹)] and other CPET variables [(ventilatory equivalent for carbon dioxide ($\dot{V}E/\dot{V}CO_2$) at AT, oxygen uptake efficiency slope and peak work rate)] were also noted.

Although the increase in AT following transfusion was statistically and significantly different, the mean difference was only 1.2 (95% CI 0.2-2.2) ml kg⁻¹ min⁻¹. Whether this would constitute a meaningful and clinically significant change remains to be confirmed but is nonetheless an encouraging finding given the wider perioperative context in which this paper was conducted and written. In the perioperative setting, optimising Hb and physical fitness (improving AT and $\dot{V}O_2$ peak) in patients prior to surgery may reduce perioperative risk and improve surgical outcome. This is certainly the founding hypothesis underpinning 'prehabilitation' before major surgery, although improvements in patient outcomes remain to be confirmed with adequately powered, randomised controlled trials, something that the PREVENTT: preoperative intravenous iron to treat anaemia in major surgery trial [149] aims to address in the context of preoperative anaemia. In the context of preoperative physical fitness, trials are planned to determine whether improving $\dot{V}O_2$ at AT following structured in-hospital exercise training leads to improved overall survival following major cancer surgery [255].

In the study by Wright and colleagues [254], it would also have been of interest to quantify the change in tHb-mass, blood and plasma volumes after transfusion in addition to the change in [Hb], as the measurement of tHb-mass may allow changes in Hb following intervention to be measured with greater precision than relying on [Hb] alone. In addition, the relative contribution of tHb-mass and

plasma volume to the change in measured [Hb] post transfusion could also have been elucidated, which may have been of use in those patients where the change in [Hb] was small and in those where AT failed to increase. Future studies may wish to use tHb-mass in this context as the primary haematological outcome variable.

2.7.3 Are changes in total haemoglobin mass associated with alterations to aerobic capacity?

A stronger relationship is consistently observed between markers of cardiorespiratory fitness and tHb-mass than with either blood volume or [Hb] [256] in athletes and healthy volunteers. Procedures to increase tHb-mass result in elevated $\dot{V}O_{2max}$, whereas the opposite is true when tHb-mass is reduced [250].

Karpovich and Millman in 1942 [257] were first to present data linking tHb-mass to exercise performance, specifically that a reduction in tHb-mass impaired athletic performance. After blood donation of 500 ml, performance during cycle ergometry dropped considerably and remained depressed for 3 weeks before recovering to pre-donation performance levels. The same authors [260] highlighted that this effect may be greatest in endurance athletes than in athletes performing shorter duration events (i.e. with a greater reliance on anaerobic energy systems). For example, when blood donation occurred in a long-distance swimmer and cross-country runner their performance was markedly reduced whereas performance in two sprint-based athletes equalled their best within a few hours after blood donation.

Similarly, another early study by Kjellberg and colleagues [258] described the relationship between BV, tHb-mass and aerobic performance in individuals of varying levels of training status. BV and tHb-mass were lower in untrained males (BV 75 ml·kg⁻¹ and tHb-mass 11.5 g·kg⁻¹) compared to moderately trained athletes (BV 90 ml·kg⁻¹ and tHb-mass 13.6 g·kg⁻¹) and elite athletes (BV 103 ml·kg⁻¹ and tHb-mass 15.7 g·kg⁻¹), respectively. The same trend was observed in females, albeit at an expected lower absolute level (approximately 10% in

this case). If tHb-mass is lowest in untrained individuals, who are otherwise healthy yet sedentary, would it be expected that in individuals with pathophysiology for tHb-mass to be lower still when compared to 'normal' individuals?

Subsequently, a close association between tHb-mass and $\dot{V}O_{2\max}$ ($r= 0.97$) was observed in the early 1950's by Astrand [259], where differences in maximal aerobic capacity between adults and children and between men and women were related to differences in total Hb (see Figure 2-4). This initial investigation laid the foundation for much of the subsequent work in relation to total body Hb and aerobic capacity.

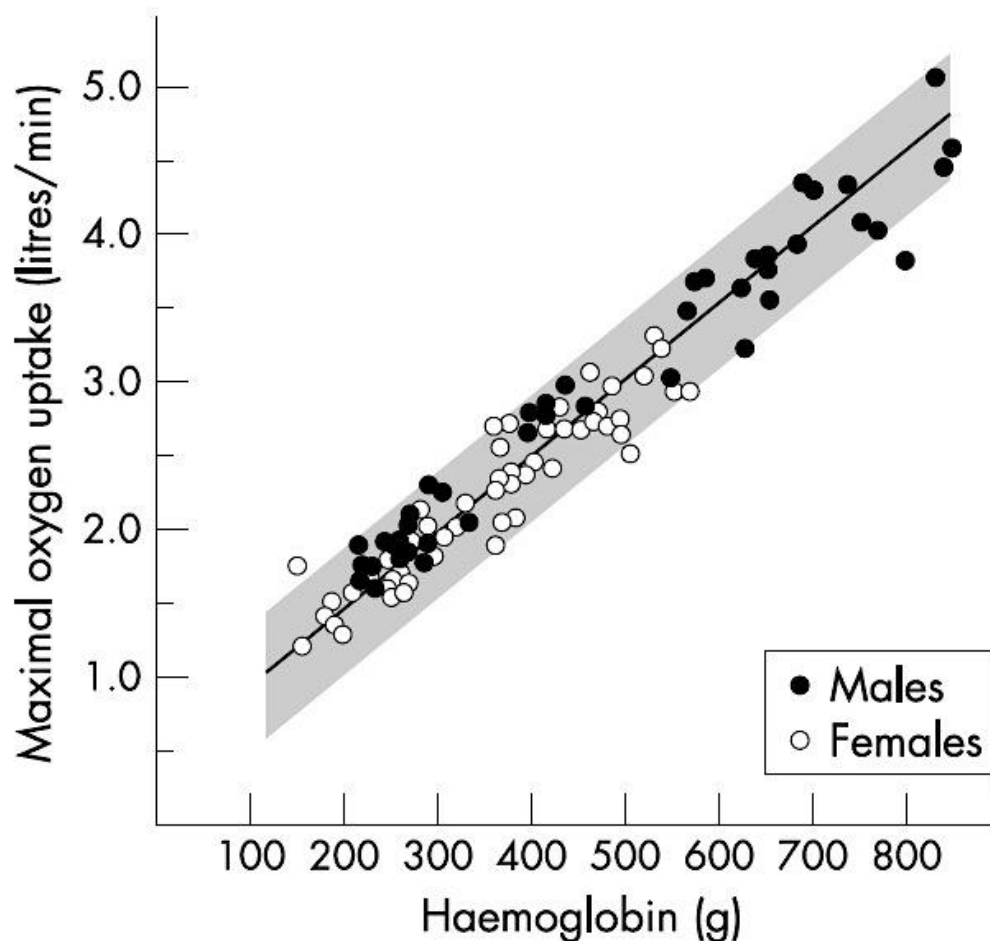


Figure 2-4. Relationship between total body haemoglobin (between 100 to 900 g) and absolute maximal oxygen consumption ($\dot{V}O_{2\max}$) in 94 individuals aged 7-30 years [259]. Figure taken from [260].

In a study assessing BV and [Hb] as determinants of maximal aerobic power, Kanstrup and Ekblom [250] utilised the chromium (^{51}Cr) labelled erythrocyte technique to quantify RCV in addition to measuring BV, PV, [Hb] and Hct. Results suggested that an elevation in $\dot{V}\text{O}_{2\text{max}}$ and physical performance may be obtained by an increase in the product of [Hb] and BV, being the tHb-mass. Decreased [Hb] in the presence of an unchanged BV (lower O_2 carrying capacity) reduces $\dot{V}\text{O}_{2\text{max}}$ and exercise performance [215] and emphasises the importance of tHb-mass as a determinant of O_2 carrying capacity and $\dot{V}\text{O}_{2\text{max}}$.

A number of other cross-sectional studies have demonstrated a strong positive association between $\dot{V}\text{O}_{2\text{max}}$ and tHb-mass including Gore and colleagues [29] who studied a cohort of trained athletes: female rowers ($n=17$, $r=0.92$, $p<0.0001$), male rowers ($n=12$, $r=0.79$, $p<0.005$) and male runners ($n=33$, $r=0.48$, $p=0.005$), and Heinicke et al [261] who investigated BV and tHb-mass in elite athletes of different disciplines (downhill skiing, swimming, running, triathlon, junior and professional cycling). $\dot{V}\text{O}_{2\text{max}}$ was significantly related to tHb-mass not only in the whole group but also in all endurance disciplines. When tHb-mass has been increased through the use of rhEPO, reciprocal increases in $\dot{V}\text{O}_{2\text{max}}$ have been reported. Specifically, $\dot{V}\text{O}_{2\text{max}}$ increased by 6-7% in 27 recreational athletes after an increase in tHb-mass of 7-12% after rhEPO use [262]. Similarly, a recent study in 19 trained men showed an improved 3,000 m running time trial performance ($11:08 \pm 1:15$ min:sec to $10:30 \pm 1:07$ min:sec, $p<0.001$) following 4 weeks of rhEPO administration. This improved performance coincided with a rhEPO-induced increase in $\dot{V}\text{O}_{2\text{max}}$ (56.0 ± 6.2 ml \cdot kg $^{-1}$ min $^{-1}$ to 60.7 ± 5.8 ml \cdot kg $^{-1}$ min $^{-1}$, $p<0.001$) and tHb-mass (12.7 ± 1.2 g \cdot kg $^{-1}$ to 15.2 ± 1.5 g \cdot kg $^{-1}$, $p<0.001$) [263].

Conversely, after a 550 ml venesection of whole blood in 9 moderately trained male and female athletes, tHb-mass was reduced by on average 77 ± 21 g [264]. This was significantly associated with a reduced absolute $\dot{V}\text{O}_{2\text{max}}$ (255 ± 130 ml \cdot min $^{-1}$ one day after phlebotomy) and was still decreased on day ten (197 ± 116 ml \cdot min $^{-1}$). The authors also observed suppressed endurance performance during this period of lower tHb-mass, although this was not objectively

quantified. On a similar note, periods of inactivity or detraining have been shown to reduce tHb-mass with associated reductions in aerobic capacity [265]. This finding could have important implications for individuals exposed to prolonged periods of bed rest or those recovering from surgery where levels of physical activity are in general very low.

What can we expect a change in aerobic capacity to be from a given change in tHb-mass? This is an important question because we can predict likely improvements in functional capacity as a result of an intervention to enhance tHb-mass. Schmidt and Colleagues [266] studied 144 male athletes from various specialities with absolute $\dot{V}O_{2\max}$ values ranging from 1010 to 6320 $\text{ml}\cdot\text{min}^{-1}$ and tHb-mass from 242 g to 1453 g. The authors found that $\dot{V}O_{2\max}$ was closely related to tHb-mass over the whole physiological range indicating that a change in tHb-mass of 1 g was associated with a change in $\dot{V}O_{2\max}$ of 4.4 $\text{ml}\cdot\text{min}^{-1}$. In addition, tHb-mass was most closely related to lean body mass (LBM) with 16.8 g of Hb per 1 kg LBM. The value of 4.4 $\text{ml}\cdot\text{min}^{-1}$ is very close to that reported by Gore and colleagues [29] and to that recently reported by Schmidt and Prommer in an excellent review article in this area [209] whereby a change in tHb-mass of 1 g was associated with a change in $\dot{V}O_{2\max}$ of approximately 4 $\text{ml}\cdot\text{min}^{-1}$.

Undertaking a meta-analysis to understand the dependency of $\dot{V}O_{2\max}$ on tHb-mass and BV, Schmidt and Prommer [256] pooled data from 611 subjects. $\dot{V}O_{2\max}$ was determined using either incremental cycle ergometry or treadmill running, with values obtained from treadmill exercise reduced by 7% to account for the greater muscle mass utilised compared to cycling. tHb-mass was measured in all subjects using the oCOR method (described in detail in Chapter 3 of this thesis). Results from linear regression revealed that a change in tHb-mass by 1 $\text{g}\cdot\text{kg}^{-1}$ was associated with a change in $\dot{V}O_{2\max}$ of 4.4 $\text{ml}\cdot\text{kg}^{-1}\text{min}^{-1}$ (males 4.2 $\text{ml}\cdot\text{kg}^{-1}\text{min}^{-1}$, females 4.6 $\text{ml}\cdot\text{kg}^{-1}\text{min}^{-1}$), showing a strong relationship ($r = 0.79$). For BV, a similar close relationship ($r = 0.76$) was observed whereby a change in BV of 1 $\text{ml}\cdot\text{kg}^{-1}$ was related to a change in $\dot{V}O_{2\max}$ of 0.7 $\text{ml}\cdot\text{kg}^{-1}\text{min}^{-1}$, in keeping with previous work by Convertino that showed a similar relationship between BV and $\dot{V}O_{2\max}$ ($r = 0.78$) [267]. Interestingly, no significant

relationship between $\dot{V}O_{2\max}$ and [Hb] (males, $r = 0.03$, females, $r = 0.12$) or haematocrit (males, $r = 0.08$, females, $r = 0.11$) was observed. The stronger relationship between tHb-mass and $\dot{V}O_{2\max}$ than other haematological markers highlights that fact that a high tHb-mass is an important determinant of a high aerobic capacity and that alterations in tHb-mass have the potential to change $\dot{V}O_{2\max}$ and potentially submaximal indices of physical fitness.

2.7.4 Clinical applications of measuring total haemoglobin mass and associations with exercise capacity

A dearth of literature exists at present that has measured tHb-mass in the clinical setting and investigated its association with markers of cardiorespiratory fitness. Research studies that have quantified tHb-mass and markers of physical fitness have studied patients with renal failure [268, 269], type 1 diabetes mellitus (T1DM) [49] and COPD [270]. The measurement of tHb-mass has also been reported in patients with CHF [96], polycythaemia [48] and patients with stable coronary artery disease [50], although not in relation to physical fitness. I will briefly discuss those studies that have assessed tHb-mass and objectively quantified physical fitness in the clinical context.

Clyne and colleagues [268] prospectively studied 20 predialytic uraemic patients aged 43 ± 12 years. Nine patients were tested on two occasions; the second testing being carried out after an average of 17 ± 5 months. The study aimed to assess the factors limiting working capacity in this patient group. Exercise testing comprised two cycle ergometry tests; firstly, a test to measure maximal aerobic capacity using a stepwise increase in work rate (10-20 watts per minute) and secondly a series of steady state work rate increments (of 20-50 watts) that increased every 4 minutes to facilitate the measurement of blood lactate levels in capillary blood. tHb-mass was determined by the alveolar carbon monoxide method [271] and BV derived from tHb-mass and [Hb]. Markers relating to chronic kidney disease were also measured and included the estimation of glomerular filtration rate (eGFR), serum calcium, phosphate, albumin and parathyroid hormone concentration (PTH). Overall, maximal exercise capacity was impaired in patients with moderate to severe uraemia

compared to age and sex-matched healthy reference values. Of particular interest in this study were those patients monitored longitudinally. A decrease in steady state exercise capacity of almost 20% was observed between the first and second tests with this impairment being associated with an earlier blood lactate accumulation (indicative of an earlier reliance on anaerobic metabolism) and a reduction in tHb-mass as uraemia progressed.

Similar findings were later reported by the same research group showing that a progressive decline in renal function induced a gradual decrease in tHb-mass and exercise capacity [269]. Specifically, 58 patients (45 ± 12 years) were studied with an eGFR ranging from 3 to 32 ml min^{-1} , allowing the effects of a progressive decline in renal function on tHb-mass and exercise capacity to be studied. tHb-mass and eGFR were partially correlated (adjusting for age and sex), $r = 0.39$, $p < 0.005$; with maximal exercise capacity and tHb-mass showing a significant, albeit weak partial correlation (adjusting for age, sex and eGFR), $r = 0.27$, $p < 0.05$. The authors commented that tHb-mass was measured rather than [Hb] or blood volume to avoid the possible effects of hypervolemia or diuretic therapy related hypovolemia. Clyne and colleagues [269] concluded that anaemia appears to contribute towards the observed reduction in maximal exercise capacity in the cohort of patients studied.

Kaponen and colleagues set out to answer two study questions in men with T1DM, firstly whether patients with T1DM (with normal kidney function) have similar blood O_2 carrying capacity (defined by [Hb], tHb-mass and RCV) compared to controls, and secondly whether O_2 carrying capacity influences $\dot{\text{V}}\text{O}_{2\text{max}}$ similarly in diabetic patients compared to controls? A similar [Hb] in patients with T1DM (14.4 g dl^{-1}) and controls (14.5 g dl^{-1}) was found, but lower tHb-mass ($10 \text{ g}\cdot\text{kg}^{-1}$ vs. $11 \text{ g}\cdot\text{kg}^{-1}$), BV (77 vs. $84 \text{ ml}\cdot\text{kg}^{-1}$) and $\dot{\text{V}}\text{O}_{2\text{max}}$ ($35 \text{ ml}\cdot\text{kg}^{-1}\text{min}^{-1}$ vs. $45 \text{ ml}\cdot\text{kg}^{-1}\text{min}^{-1}$) in the T1DM group. In patients with T1DM both tHb-mass (51%) and blood volume (45%) explained a large proportion of the variance in $\dot{\text{V}}\text{O}_{2\text{max}}$ and when compared to age- and leisure-time physical activity matched controls showed a shallower linear regression slope between $\dot{\text{V}}\text{O}_{2\text{max}}$ and tHb-mass ($2.4 \text{ ml}\cdot\text{kg}^{-1}\text{min}^{-1}$ vs. $3.6 \text{ ml}\cdot\text{kg}^{-1}\text{min}^{-1}$, respectively). Therefore, for a given tHb-mass and BV, patients with T1DM had a reduced $\dot{\text{V}}\text{O}_2$ compared to controls. This study appears to confirm that the

measurement of tHb-mass may provide useful additional information as an early and sensitive marker of blood O₂ carrying capacity than measuring [Hb] alone, given that there were no differences in [Hb] between the T1DM and control groups [49].

In patients with COPD, repeated exposure to short-term hypoxia, termed 'interval hypoxia' provides a potential therapeutic intervention to increase exercise tolerance. Burtcher and colleagues [270] provided evidence that improvements in exercise tolerance may in part be related to changes in tHb-mass following such intermittent hypoxic exposure. In a double-blinded, randomised controlled trial in eighteen patients at risk of, or with mild COPD (aged 33-72 years), tHb-mass increased by 4%, and was positively related to a significant change in total exercise time (+10% increase, $r = 0.59$), time to reach AT during CPET (+13% increase) and the change in $\dot{V}O_2$ peak ($r = 0.49$) following a 3-week intervention of intermittent hypoxic exposure (totalling 15 sessions). Such hypoxic exposure is commonplace in endurance athletes who regularly attend altitude training camps and/or sleep in altitude tents to mimic the effects of hypoxia whilst the athlete remains at sea level. The results of the study by Burtcher and colleagues [270] are promising and may have wider implications to a range of patient populations where increasing tHb-mass and physical fitness may be advantageous (e.g. prior to major surgery). In addition, this study adds further evidence highlighting the benefits of measuring tHb-mass in conjunction with more routine haematological markers of O₂ carrying capacity such as [Hb] and Hct, which showed no significant change in either the hypoxic or normoxic group in this study following intervention.

The result of Burtcher and colleagues [270] and also those of Kaponen et al [49] raise the following discussion. If [Hb] alone had been used to define O₂ carrying capacity, then it would have been assumed unchanged ([Hb] of around 145 g·l⁻¹) in both patients with T1DM and healthy controls in the study by Kaponen and colleagues and 145 g·l⁻¹ vs 146 g·l⁻¹ pre-and post-hypoxic exposure in the study by Burtcher and colleagues. Thus, deficiencies in blood O₂ carrying capacity may be masked if [Hb] is used in isolation due to compartmental fluid shifts confounding results. [Hb] is determined by tHb-mass and circulating PV, thus a substantial reduction in O₂ carrying capacity, related

to a low tHb-mass, may thus be masked if PV is contracted, as may be the case in many disease states. Similarly, increases in PV may decrease [Hb] even in the context of a normal tHb-mass. Therefore, without knowledge of tHb-mass and PV, our interpretation of [Hb] may be limited.

Chapter 3

3 General methodology

Chapter three outlines the methods I have used during this thesis. Firstly, I begin with a thorough overview of the oCOR method, in particular the specific equipment required, the procedures involved, blood sampling and the subsequent calculation of tHb-mass. Despite being the most widely used method to quantify tHb-mass in sports medicine, the oCOR method has been rarely applied in the clinical setting. I use the oCOR method in Chapters 4, 5, and 6 which follow. Secondly, I provide a detailed description of the methods relating to CPET, its conduct, equipment, calibration and key CPET variables (AT and $\dot{V}O_2$ peak). I utilise CPET in Chapter 6 to quantify exertional $\dot{V}O_2$ at AT and $\dot{V}O_2$ peak as markers of cardiorespiratory fitness prior to surgery.

3.1 Optimised carbon monoxide rebreathing method (oCOR)

Total haemoglobin mass was determined using the optimised carbon monoxide rebreathing method described in detail by Schmidt and Prommer [19]. This method consists of a 2-minute rebreathing procedure of a known CO volume (0.5 to 0.9 ml of CO per kg of body mass in this thesis, depending on gender and clinical status, with some adjustment for [Hb], body mass index and the general status of the patient). Each participant was seated for 15 minutes prior to testing to allow stabilisation of plasma volume, after which a mouthpiece containing approximately 10 g of soda lime (carbon dioxide scrubber) connected them to a spirometer (Spico-CO Respirations-Applikator, Blood Tec, Bayreuth, Germany) and a 3-litre anaesthetic bag pre-filled with pure medical grade oxygen (see

Figure 3-1). Thereafter the patient completely exhaled to residual volume and was then instructed to take a deep breath in through the spirometer as the CO dose was administered via a pre-filled 100 ml syringe. Consequently, all the CO was inhaled in the first fraction of the breath and subsequently distributed within the alveoli. Given that the affinity between Hb and CO is some 220- to 240- times greater than that of Hb and O_2 [119], the majority of CO will diffuse

into the bloodstream within the first few seconds after inhalation. To further support the diffusion of CO into the bloodstream, patients held their breath for 10 seconds after the first inspiration, after which they continued normal breathing from the spirometer for a further 1 minute 50 seconds. The participant was disconnected after exhaling to residual volume, which is necessary to quantify the CO not absorbed into the bloodstream. Finally, participants fully exhaled to residual volume into a CO gas analyser with parts per million sensitivity (Dräger Pac 7000, Drägerwerk AG & Co. KGaA, Bayreuth, Germany) before and at minute 4 after CO rebreathing to quantify the CO that was not taken up by the body. In addition, to confirm that there was no gas leakage during rebreathing, the portable CO monitor was placed near the nose, mouth and spirometer throughout the 2-minute rebreathing phase.

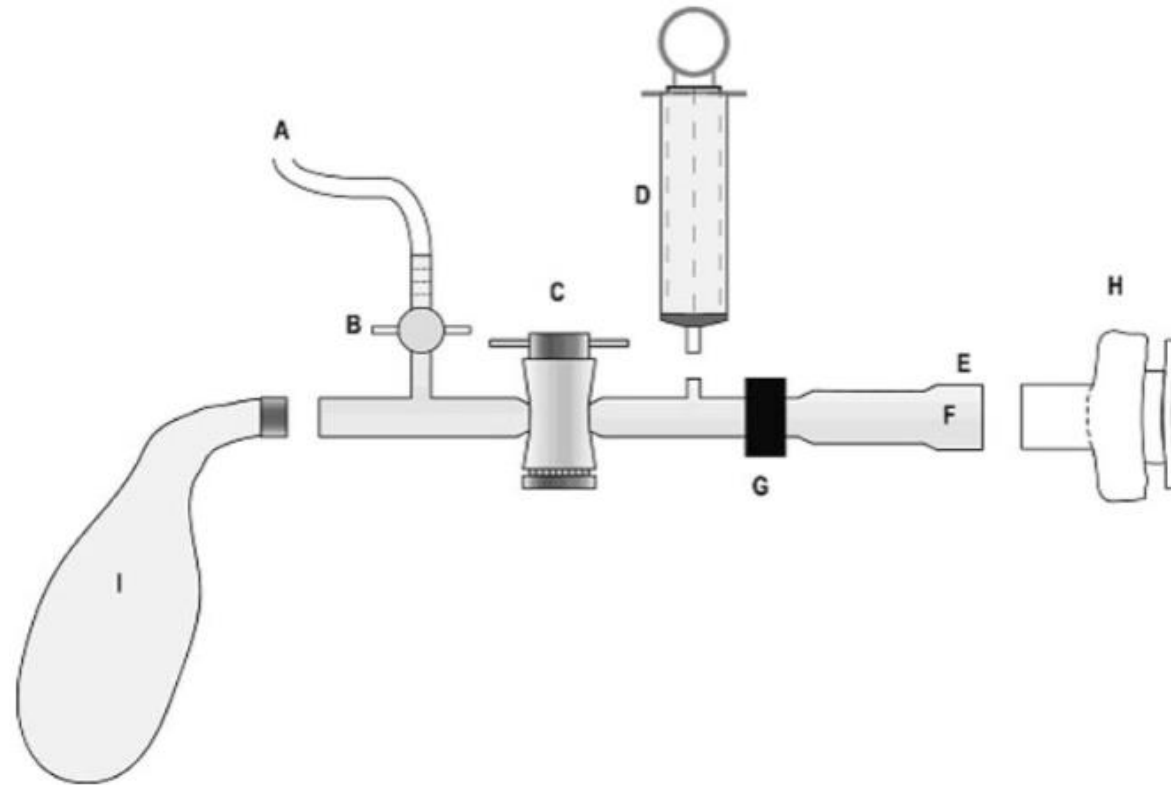


Figure 3-1. Custom made spirometer developed for the optimised carbon monoxide rebreathing method by Schmidt and Prommer. (A) oxygen (O_2) tube, (B) O_2 valve, (C) valve of the O_2 reservoir, (D) prefilled CO syringe (100 ml capacity), (E) adapter to enable mouth piece connection, (F) netting bag of soda lime (CO_2 scrubber), (G) sleeve, (H) mouth piece, (I) 3 litre anaesthetic bag (containing pure medical grade O_2). Taken from the original paper by Schmidt and Prommer [19]. Patent numbers: US20050075552 A1; DE10222750C1.

3.1.1 Blood sampling

At UCLH, [Hb] was collected (Hb 201 Microcuvette, Hemocue AB, Angelholm, Sweden) from a fingertip capillary blood sample and analysed immediately using a regularly calibrated (as per manufacturer's instructions) HemoCue® Hb 201+ (Hemocue AB, Angelholm, Sweden) when [Hb] could not be obtained from venous blood at the time of testing or was not available on the same day as the tHb-mass test on the hospital electronic blood records system. At UCLH, fingertip capillary samples (200 µl) were collected before and 6- and 8-min after the start of CO rebreathing (Na-heparinized 200 µl RAPIDLyte Multicap Capillary tubes, Siemens Healthcare Diagnostics Inc, Deerfield, IL, USA), with samples analysed within 15 minutes for percent carboxyhaemoglobin (%COHb) using a blood gas analyser with 0.1% precision (Hemoximeter; Cobas b 221 POC system, Roche Diagnostics Ltd, Rotkreuz, Switzerland). At Southampton, [Hb] was measured in venous blood. An intravenous cannula was inserted into the dorsal network of the hand prior to tHb-mass testing at Southampton, allowing venous blood samples (200 µl) to be collected before and at 6- and 8-mins after CO rebreathing via a Na-heparinized blood gas syringe (RAPIDLyte, Siemens Healthcare Diagnostics Inc, Deerfield, IL, USA). Percent carboxyhaemoglobin was determined with 0.1 % precision at Southampton using the RAPIDPoint 500 Blood Gas System (Siemens Healthcare Diagnostics Inc, Deerfield, IL, USA). Blood sampling from venous, arterial and capillary blood has been shown to yield an identical $\Delta\%COHb$ and therefore identical tHb-mass values [272]. All blood samples for the determination of [Hb], Hct and %COHb were collected under the same conditions, from the same anatomical site, with patients in a seated position. Blood samples were analysed within 10-15 minutes of one another due to %COHb being analysed after the oCOR test using a blood gas machine (which takes 15 minutes).

Venous blood samples were taken if there were no recent full blood test results on site specific hospital electronic records systems, and if patients provided additional (and optional) consent for venous blood samples to be collected. Where appropriate a butterfly needle (BD winged infusion set, Becton, Dickinson, Oxford, UK) was inserted into an antecubital, cephalic or vein

located in the dorsal network of the hand. Blood samples (3-4 ml) were collected in two BD Vacutainers, drawn in the correct and specific order, which prevents the contamination of additives between different tubes; firstly, a serum-separating tube (SST, gold top) containing silica and polymer gel was filled, after which a vacutainer (purple top) containing ethylenediaminetetraacetic acid (EDTA 1.8 mg per ml of blood) was drawn. After blood samples were collected they were repeatedly inverted to ensure thorough mixing of additives and then allowed to clot at room temperature for 20-30 minutes prior to centrifugation. Thereafter, the SST Vacutainer was centrifuged at 4 degrees for 20 minutes at 2500 revolutions per minute using a refrigerated laboratory centrifuge (Rotofix 32 A, Hettich Zentrifugen, Tuttlingen, Germany). Subsequently, the resulting supernatant (designated as serum) was transferred immediately using a disposable pipette into appropriately labelled 0.5-1.0 ml aliquots and stored in a -80°C freezer until future analysis.

3.1.2 Calculation of tHb-mass

tHb-mass was calculated using a specifically designed Excel spreadsheet (Microsoft Excel 2011 for Apple Macintosh) based on the formula below:

$$\text{tHb-mass (g)} = K \times \text{MCO(ml)} \times 100 \times (\Delta\% \text{COHb} \times 1.39)$$

$$K = \text{current barometric pressure} \times 760^{-1} \times [1(0.003661 \times \text{current temperature})]$$

$$\text{MCO} = \text{CO}_{\text{adm}} - (\text{CO}_{\text{system + lung (after disconnection)}} + \text{CO}_{\text{exhaled (after disconnection)}})$$

$$\text{CO}_{\text{adm}} = \text{CO volume administered into the system}$$

$$\text{CO}_{\text{system + lung (after disconnection)}} = \text{CO concentration in spirometer} \times (\text{spirometer volume} + \text{remaining volume in the lung after rebreathing})$$

$$\text{CO}_{\text{exhaled (after disconnection)}} = \text{end-tidal CO concentration} \times \text{alveolar ventilation} \times \text{time}$$

$$\Delta\% \text{COHb} = \text{difference between baseline \%COHb and \%COHb post CO administration (average of 6- and 8-min \%COHb values)}$$

$$1.39 = \text{Hüfners number (constant) (ml CO} \times \text{g} \cdot \text{Hb}^{-1})$$

Where CO, carbon monoxide; %COHb, percent carboxyhaemoglobin; lung residual volume, 1500 ml in men, 1200 ml in women; alveolar ventilation, 5000 ml·min⁻¹.

Blood volume (BV), plasma volume (PV) and red cell volume (RCV) were calculated from MCHC, [Hb] and tHb-mass, as follows:

$$BV \text{ (ml)} = tHb\text{-mass (g)} / [Hb] \text{ (g·dl}^{-1}) \cdot 100$$

$$RCV \text{ (ml)} = tHb\text{-mass (g)} / MCHC \cdot 100$$

$$PV \text{ (ml)} = BV - RCV$$

When haematocrit and haemoglobin concentration values were obtained from capillary blood at UCLH these values were corrected to venous conditions using the following formulas [27, 273]:

$$[Hb] \text{ (g·dl}^{-1}) = [Hb_{capillary}] \cdot 0.8787 + 1.24$$

$$Hct \text{ (\%)} = [Hct_{capillary}] \cdot 0.8425 + 5.23$$

Example: If 70 ml of CO is inhaled (based on a 70-kg athletic male, using the 1 ml of CO per kg body mass ratio), per Hüfner's constant this will bind to 50 g of Hb. If the measured $\Delta\%$ COHb following CO rebreathing is 6% (i.e. 6% of total haemoglobin is bound to CO), then 6% of tHb-mass is 50 g and tHb-mass is 833 g or 11.9 g·kg⁻¹. This example is based on the volume of CO in the blood and the change in percent carboxyhaemoglobin in blood pre-and post-rebreathing only, and does not consider the CO that is not absorbed into the blood stream as described in more detail in the methods and calculation of tHb-mass above.

3.2 Cardiopulmonary exercise testing (CPET)

Prior to undergoing CPET, I took a medical history for each patient to rule out contraindications to CPET (see 3.2.4) as well as recording current medications. Patients were instructed to take medications as usual on the day of testing. In addition, spirometry was performed to measure forced vital capacity (FVC), forced expiratory volume in the first second (FEV₁) and the FEV₁/FVC ratio using an Easy on-PC spirometry device (New Diagnostic Design, Medical Technologies, Inc, Andover, MA, USA). Pre CPET [Hb] was also collected (Hb 201 Microcuvette, Hemocue AB, Angelholm, Sweden) from a fingertip capillary blood sample and analysed immediately (HemoCue® Hb 201+, Hemocue AB, Angelholm, Sweden) as per local standard operating procedures, if [Hb] was not available on electronic records systems on the same day as CPET.

CPET was performed according to the American Thoracic Society/American College of Chest Physicians (ATS/ACCP) guidelines [274], under stable environmental conditions (temperature and barometric pressure), with continuous 12-lead electrocardiogram monitoring and in the presence of an experienced clinical exercise physiologist and clinician (where appropriate). Additionally, blood pressure was monitored once at rest and every two minutes during exercise with patients wearing a blood pressure cuff (Orbit-K cuff, SunTech Medical, Morrisville, USA) connected to a Multilyzer (Cortex Biophysik, Leipzig, Germany) with integrated fingertip pulse oximetry to monitor blood O₂ saturation. Patients were instructed to abstain from caffeine and nicotine on the day of CPET and be fasted for at least 1.5 hours prior to exercise.

3.2.1 CPET equipment and calibration

At UCLH, gas analysis was performed using the Metalyser 3B-R2 breath-by-breath system (Cortex Biophysik, Leipzig, Germany), which allows the continuous measurement of volume and concomitantly determines inspired and expired O₂ and CO₂. At Southampton, gas analysis was performed using the

Ergostik metabolic unit [(Geratherm Respiratory GmbH (Love Medical Ltd, Manchester, UK)]. Breath-by-breath oxygen uptake ($\dot{V}O_2$) and carbon dioxide output ($\dot{V}CO_2$) were recorded, together with respiratory rate, tidal volume, minute ventilation and end-tidal gas tensions.

At both UCLH and Southampton, bi-daily calibration of the pressure analyser (am and pm) were performed on testing days using a digital barometer (GPB 3300 Digital Barometer, GHM Messtechnik GmbH Standort Greisinger, Regenstauf, Germany). Prior to every CPET, gas calibration of the O_2 and CO_2 analyser and volume transducer calibration were performed. All calibration values were recorded in a calibration log. Specific details of CPET calibration procedures are described below.

3.2.2 Gas calibration

The Cortex Metalyser 3B-R2 breath-by-breath metabolic cart was switched on at least 45 minutes prior to CPET to allow sufficient time for the equipment to warm up as per manufacturers recommendations. Subsequently a two-point gas calibration was carried out using two gas mixtures; Gas 1 (ambient air: 0.03% CO_2 , 20.93% O_2) and Gas 2 (calibration/reference gas: 5% CO_2 , 15% O_2), after which values were checked to ensure they were within an acceptable error range (ambient air: O_2 $20.93 \pm 0.05\%$, CO_2 $0.03 \pm 0.02\%$; calibration gas: O_2 $15 \pm 0.05\%$, CO_2 $5 \pm 0.05\%$) as set out in the ATS/ACCP guidelines [274]. If checked gas values fell outside of these limits the gas calibration procedure was re-run from the start until values fell within these accepted ranges of error.

3.2.3 Triple V ML II Volume transducer calibration

The Triple V volume transducer (Cortex Biophysik, Leipzig, Germany) was calibrated using a 3 litre syringe (Hans Rudolph, Inc, Shawnee, USA). Five valid inspiratory and expiratory strokes were required for successful volume calibration. Calibration values were checked to ensure they were within an acceptable range of error (3.0 ± 0.1 litres). Volume calibration checks were

carried out at 3 different flow rates to mimic different rates of ventilation that occur during an incremental exercise test, specifically 0.5, 1.5 and 3 l·min⁻¹.

3.2.4 Contraindications for CPET

Patients were screened prior to every CPET to rule out contraindications to undergoing testing. Absolute and relative contraindications to CPET were based on those outlined in the American Thoracic Society/American College of Chest Physicians (ATS/ACCP) guidelines [274], described in detail below:

3.2.4.1 Absolute Contraindications to CPET

- I. Acute MI (within 7-10 days of transmural infarct or within 5 days if minor and uncomplicated)
- II. Uncontrolled arrhythmias causing symptoms or haemodynamic compromise
- III. Severe left main stem stenotic lesions.
- IV. Uncontrolled hypertension (or systolic BP above 180 mmHg at rest)
- V. Pulmonary hypertension
- VI. Pulmonary oedema
- VII. Room air desaturation at rest (less than 85%)
- VIII. Acute inflammatory conditions (pericarditis, myocarditis)
- IX. Unstable angina (patient pain-free for 4 days before test)
- X. Dissecting aneurysm
- XI. Acute or recent pyrexial illness
- XII. Thyrotoxicosis
- XIII. Syncope
- XIV. Thrombosis of lower extremities

3.2.4.2 Relative Contraindications to CPET

- I. Moderate stenotic valvular heart disease
- II. Left main coronary stenosis or its equivalent
- III. Frequent/fast atrial/ventricular rhythms
- IV. Hypertrophic obstructive cardiomyopathy
- V. Left ventricular failure/congestive cardiac failure
- VI. Complete heart block
- VII. Left ventricular hypertrophy
- VIII. Bundle branch block
- IX. Orthopaedic/neurological impairment
- X. Elderly, frail or disabled patients
- XI. Advanced or complicated pregnancy

3.2.5 CPET protocol

Patients pedalled an electromagnetically-braked cycle ergometer (UCLH, Lode Corival BV, Groningen, Netherlands; Southampton, Ergoline 2000, Ergoline GmbH, Bitz, Germany).

A 3-minute rest period followed fitting of relevant equipment, after which unloaded cycling was performed at a cadence of 60 to 65 rpm for 3 minutes. Thereafter, patients performed a symptom-limited continuous incremental exercise ramp protocol, determined by the physiologist or clinician on the basis of predictive work rate algorithms and patient-reported activity levels [77]. Ramp gradient was set to 10-25 W min⁻¹ based on patient height, weight and age, with the aim being a test duration of between 8 to 12 minutes. Tests were terminated at patient exhaustion, or until the patient was unable to maintain a cadence of 40 rpm for more than 30 seconds despite verbal encouragement. The clinician stopped the test if the patient developed a sign or symptom listed in the ATS/ACCP CPET guidelines, which included: new arrhythmia; more than 2 mm of ST elevation or depression on the ECG; an arterial blood pressure of more than 250 mm Hg systolic or 120 mm Hg diastolic (see 2003 ATS/ACCP statement on Cardiopulmonary Exercise Testing for an exhaustive list [274])

Following termination of CPET, patients were encouraged to perform a 'cool-down' period of unloaded cycling for 3-5 minutes.

3.2.6 Determination of primary CPET variables

The two main variables measured during CPET to quantify cardiorespiratory fitness are the $\dot{V}O_2$ peak and $\dot{V}O_2$ at AT. Below outlines in detail how both fitness markers were determined after CPET in each patient.

3.2.6.1 Peak oxygen consumption ($\dot{V}O_2$ peak)

Aerobic capacity was quantified by measuring the peak exertional oxygen consumption ($\dot{V}O_2$ peak, normalised for body weight and thus expressed in $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) achieved on a symptom limited, maximal incremental ramp test. Thus, $\dot{V}O_2$ peak reflects a patient's limit of tolerance and providing sufficient volition/effort is closely reflective of $\dot{V}O_2$ max in the absence of a plateau in $\dot{V}O_2$ as per the classical definition. $\dot{V}O_2$ peak was recorded as the highest average $\dot{V}O_2$ over the final 30s of exercise [275] and is shown in Figure 3-2 (panel 3 of the nine panel plot) by the black arrow.

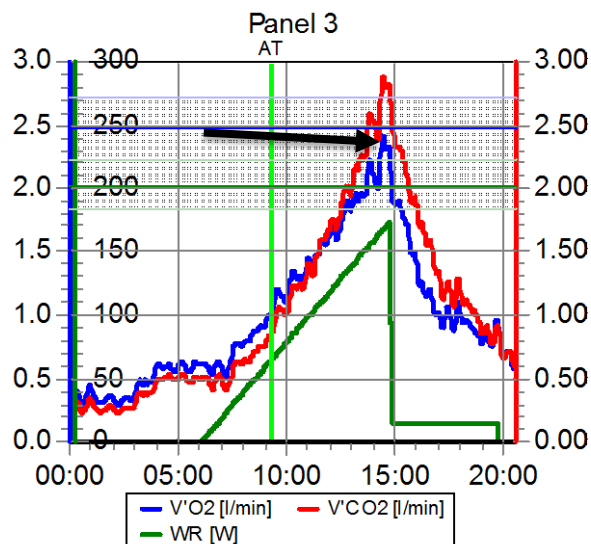


Figure 3-2. Oxygen consumption (blue line) and carbon dioxide output (red line) during incremental exercise. Dark green line represents the linear increase in work rate during the incremental ramp test. $\dot{V}O_2$ peak is shown by the black arrow. Screen shot taken from real patient test using Metasoft version 3.9 (Cortex Biophysik, Leipzig, Germany).

3.2.6.2 Anaerobic threshold (AT)

The $\dot{V}O_2$ at anaerobic threshold ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) was determined by myself at UCLH and by an exercise physiologist at Southampton, both verified by a consultant physician with experience in conducting and interpreting CPET. Middle five of seven averaging of the breath-by-breath data was performed prior to determining the AT as per normal practice. Specifically, AT was determined using a combination of the modified V-slope [276], ventilatory equivalents and end-tidal pressure methods shown in Figure 3-3 [277], which improves the rigor of AT detection [278]. In detail, the AT can be seen plotted in Figure 3-3 represented by a vertical green line on panels 5, 6, 8 and 9. Panel 5 shows the relationship between $\dot{V}O_2$ (x-axis) and $\dot{V}CO_2$ on the y-axis. At the AT we see an excess $\dot{V}CO_2$ output compared to ongoing $\dot{V}O_2$, which is illustrated by a break-away from the black dotted line (denotes a 1:1 relationship between $\dot{V}O_2$ and $\dot{V}CO_2$). The excess $\dot{V}CO_2$ reflects the additional $\dot{V}CO_2$ released from bicarbonate (HCO_3^-) buffering of protons (H^+) associated with the accumulation of lactate [279] with increasing exercise intensity.

Panel 6 shows the AT occurring at the nadir in $\dot{V}E/\dot{V}O_2$ (blue line) before hyperventilation relative to O_2 but not CO_2 (red line, $\dot{V}E/\dot{V}CO_2$) occurs. This is also supported in panel 9 showing an increase in the end-tidal pressure for O_2 ($P_{ET}O_2$, dark blue line), with no decrease in end-tidal pressure for CO_2 ($P_{ET}CO_2$, pink line) measured at the end of exhalation (representing alveolar ventilation). The AT can also be seen plotted in panel 8, which shows the profile of the respiratory exchange ratio (RER, black line) during incremental exercise. As can be seen there is an increase in RER at the AT reflecting an excess $\dot{V}CO_2$ relative to $\dot{V}O_2$ without evidence of hyperventilation (which can affect RER independently from excess $\dot{V}CO_2$ as a by-product of increased anaerobic metabolism).

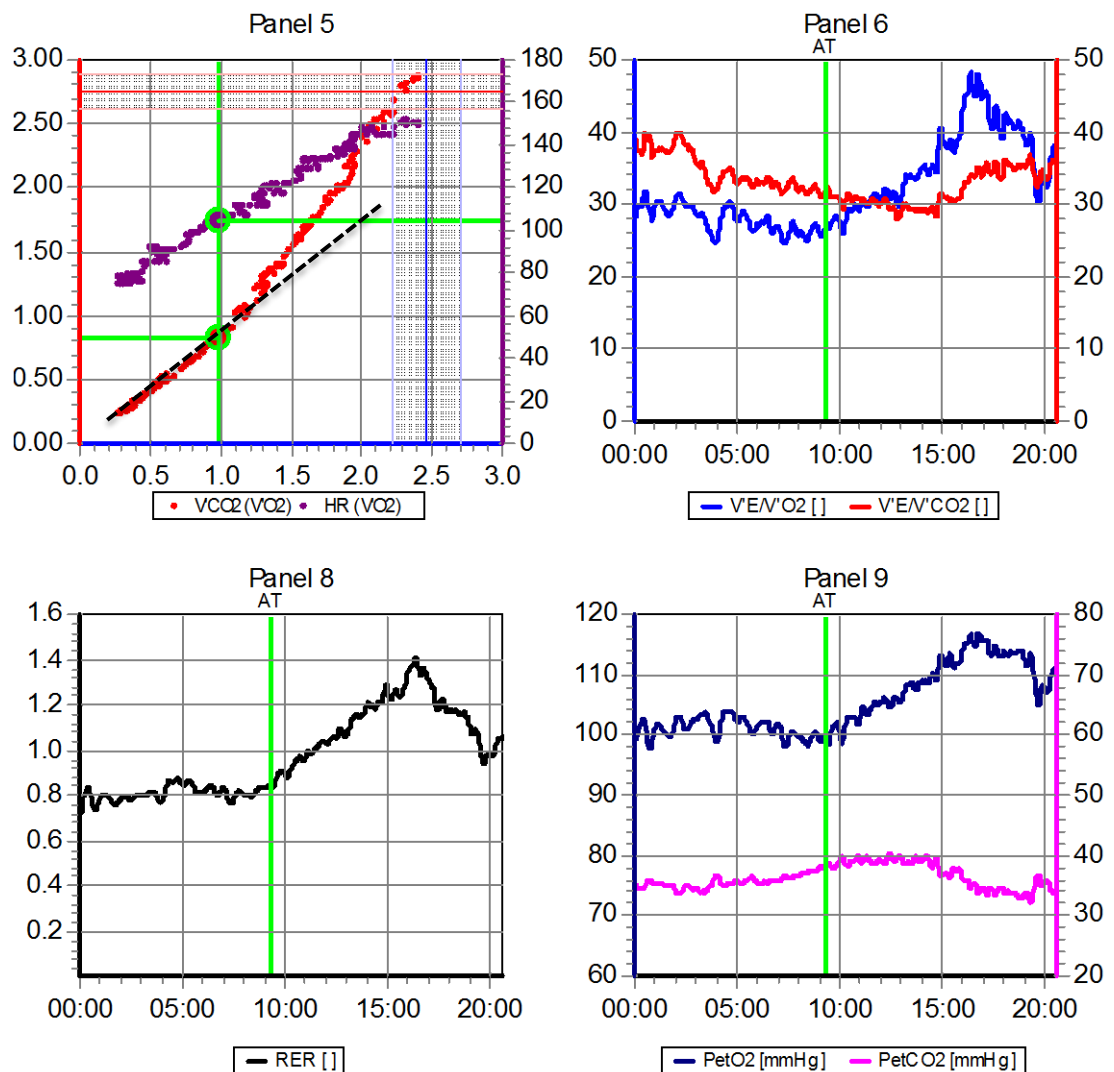


Figure 3-3. Modified V-slope (panel 5), ventilatory equivalents (panel 6), and end-tidal pressure methods (panel 9) along with a plot of respiratory exchange ratio (panel 8) used for the detection of the anaerobic threshold (AT). The green line represents the AT in all panels and is reported as the oxygen consumption ($\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) at this point. Screen shot taken from real patient test using Metasoft version 3.9 (Cortex Biophysik, Leipzig, Germany).

Chapter 4

- 4 Test retest reliability of total haemoglobin mass using the optimised carbon monoxide rebreathing method**

4.1 Abstract

Background: Total haemoglobin mass (tHb-mass) represents the absolute mass of circulating haemoglobin, which determines the oxygen transport capacity of the blood, providing valuable information in the fields of clinical and sports medicine. The optimised carbon monoxide rebreathing (oCOR) method allows precise, valid and reliable measurement of tHb-mass with low measurement error ($\leq 2\%$) reported in many institutions. However, it is important for every laboratory using the oCOR method to assess the site-specific measurement error of tHb-mass. As a result, the aim of this study was to assess the test retest measurement error of tHb-mass in our laboratory.

Methods: Ten participants (50% female) volunteered to participate in the study (age 24 ± 5 years, weight 60.7 ± 12.4 kg). Each subject performed two oCOR tests to determine tHb-mass separated by no less than 12 hours and no more than 7 days. Typical error (TE) expressed as coefficient of variation (CV %) with 95% confidence intervals (95% CI) for tHb-mass were calculated according the methods of Hopkins [280]. Bland-Altman plots assessed differences in tHb-mass between repeat tests [281].

Results: tHb-mass from test₁ (630 ± 190 g) and test₂ (631 ± 188 g) did not differ ($p = 0.594$) and were highly correlated ($r = 1.000$, $p < 0.0001$). This was also the case for weight-adjusted tHb-mass ($\text{g} \cdot \text{kg}^{-1}$) between test₁₋₂ (tHb-mass $9.3 \pm 1.6 \text{ g} \cdot \text{kg}^{-1}$ versus $9.3 \pm 1.6 \text{ g} \cdot \text{kg}^{-1}$, $p = 0.788$). TE as CV % (95 % CI) for tHb-mass values between test₁₋₂ for all patients ($n = 10$) was TE 1.9 % (95% CI 1.3-3.4 %) or 9 g (95% CI 6-16 g). Based on the mean tHb-mass of 630 g, this equates to a typical error of 12 g. Mean difference in tHb-mass between test₁ and test₂ was 1 g with 95% limits of agreement of -4 to 6 g.

Conclusions: The oCOR method to determine tHb-mass in our laboratory is reliable with a similarly low test retest measurement error compared to other established institutions.

4.2 Introduction

The oCOR method allows tHb-mass to be quantified and shows comparable measurement error to the gold standard technique (^{51}Cr labelled RBCs), but without the associated risks of using radioactive isotopes [117]. Previous studies have reported excellent reliability when using the oCOR method with an error of measurement (expressed as typical error, TE) of ~2% commonly being reported [44, 46, 282, 283]. A meta-analysis of studies using the oCOR method reported a mean measurement error of 2.2% for tHb-mass, although values ranged from 1.4-3.5% across different laboratories [117]. In addition, determination of tHb-mass by the oCOR method is dependent on the repeatable measurement of percent carboxyhaemoglobin (%COHb) in blood by a hemoximeter [284], with previous studies typically using the Radiometer OSM3 or ABL-80 devices (Radiometer Medical ApS, Denmark) [19, 284-286], whilst I used a Cobas b 221 POC Hemoximeter (Roche Diagnostics, Switzerland) as this was the analyser in use at our institution. Analyser error of $\leq 1\%$ is essential for the accurate measurement of %COHb and subsequent tHb-mass calculation.

If there is an intention or justification for the oCOR method to be used more widely in the clinical setting as both a research and clinical tool, then an appreciation of population distribution norms and what a patient's normal physiological variation is also important to establish. Given that a test cannot be valid without first meeting the criteria of reliability, it is essential for any institution implementing the oCOR method to quantify laboratory-specific measurement error in tHb-mass in the first instance. Without knowledge of measurement error (of any test or physiological variable in fact), it is often assumed that tHb-mass has been reliably determined so that if the test was repeated a few days later, a similar value would be obtained. Chapter 4 tested this assumption.

4.2.1 Study aims

- i) To quantify the test-retest typical error of total haemoglobin mass determined by the 2-minute optimised carbon monoxide rebreathing method in our institution, and
- ii) To quantify the repeatability of %COHb measurements using the Cobas b 221 POC Hemoximeter (Roche Diagnostics, Rotkreuz, Switzerland) used in our institution.

4.3 Methods

4.3.1 Ethics

The study protocol and procedures were approved by the University College London (UCL) Research Ethics Committee (Project ID: 6654/001), and conformed to the standards set by the Declaration of Helsinki. Participants were provided with an information sheet prior to written informed consent being obtained (see Appendix A and B, respectively).

4.3.2 Inclusion criteria

- I. Male or female adults (age > 18 years)
- II. Free from chronic disease

4.3.3 Exclusion criteria

- i) Smokers
- ii) Baseline %COHb > 5 %

4.3.4 Participants

Participants were employees from either University College London or University College Hospitals NHS Foundation Trust who were recruited through word of mouth and following advertisement via email correspondence. Ten healthy individuals (50% female) volunteered to participate in the study (age 24 ± 5 years, height 154.6 ± 9.5 cm, weight 60.7 ± 12.4 kg). All participants were non-smokers, and informed to refrain from strenuous activity, alcohol and caffeine intake for 24 hours before each oCOR test.

4.3.5 Study design

Twenty oCOR procedures were carried out in total with each participant performing two oCOR tests separated by no less than 12 hours and no longer than 7 days apart to allow a comparison of tHb-mass measurements. Where possible both tests were carried out at a similar time of day on an individual participant basis to minimise the effect of diurnal variations [287].

4.3.6 Statistics

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) (Version 23.0 for Apple Macintosh, Chicago, IL, USA). Values are presented as mean \pm standard deviation (SD), unless otherwise stated. Repeatability of %COHb was assessed from five capillary blood samples for the Cobas b 221 POC blood gas machine (Hemoximeter) using the coefficient of repeatability (CR). CR is calculated by multiplying the within-subject SD by 2.77 ($\sqrt{2} \times 1.96$) [288], which quantifies absolute reliability of measurement error in the same units as the measurement tool. The typical error (TE) expressed as coefficient of variance (CV %) with corresponding 95% confidence intervals (95% CI) for tHb-mass were calculated according to the methods described by Hopkins [280], where TE is calculated from the standard deviation of difference scores $\div \sqrt{2}$. To assess differences in tHb-mass between repeat tests, limits of agreement (LoA) and 95% CI were calculated by

the Bland-Altman method [281] using a specifically developed Excel spreadsheet [289]. Correlation analyses were performed using either Pearson's correlation coefficient or intraclass correlation coefficient (ICC) where appropriate. Paired t-tests were performed to assess differences in %COHb, total haemoglobin mass, blood volume, red cell volume and plasma volume between tests₁₋₂. Significance was accepted as a p-value < 0.05.

4.4 Results

4.4.1 Repeatability of the Cobas b 221 POC Hemoximeter (Roche Diagnostics, Rotkreuz Switzerland)

The coefficient of repeatability (CR) of baseline %COHb for 5 capillary blood samples measured by the Cobas b 221 POC Hemoximeter (Roche Diagnostics, Rotkreuz, Switzerland) was ± 0.14 %, see Table 4-1.

Table 4-1. Mean, standard deviation and coefficient of repeatability of percent carboxyhaemoglobin from five consecutive capillary blood samples taken from the same participant.

Sample	%COHb
1	0.80
2	0.70
3	0.70
4	0.80
5	0.70
Mean	0.74
SD	0.05
CR	± 0.14

%COHb, percent carboxyhaemoglobin; CR, coefficient of repeatability.

4.4.2 Reliability of haemoglobin concentration and haematocrit

Table 4-2. Test retest reliability of haemoglobin concentration and haematocrit.

ID number	[Hb] (g·l ⁻¹) T1	[Hb] (g·l ⁻¹) T2	Hct (%) T1	Hct (%) T2
TRT01 (M)	141.6	144.2	42.9	41.0
TRT02 (M)	137.2	138.1	42.3	43.4
TRT03 (F)	100.3	106.4	35.2	34.8
TRT04 (F)	115.2	122.2	39.2	39.6
TRT05 (M)	146.0	155.6	44.3	41.8
TRT06 (F)	113.5	123.1	38.1	37.2
TRT07 (F)	109.9	112.6	36.4	35.9
TRT08 (F)	128.4	123.1	38.5	38.8
TRT09 (M)	145.1	133.7	42.0	42.9
TRT10 (M)	148.6	146.8	45.9	44.9
Mean	128.6	130.6	40.5	40.0
SD	17.6	15.7	3.5	3.4

There were no differences in either [Hb] or Hct (%) between oCOR test₁₋₂, see Table 4-2. Repeat [Hb] and Hct (%) values were strongly correlated ([Hb] ICC $r = 0.94$ (95% CI 0.77-0.98), $p < 0.0001$; Hct (%) ICC $r = 0.96$ (95% CI 0.84-0.99), $p < 0.0001$). Typical error for Hct (%) between test₁₋₂ was TE 4.0% (95% CI 2.7-7.3) and for [Hb] TE 7.5 % (95% CI 5.1-13.9). The mean difference (SD) for [Hb] and Hct (%) between tests was $2.0 \pm 6.7 \text{ g·l}^{-1}$ and $-0.46 \pm 1.7\%$, respectively.

4.4.3 Percent carboxyhaemoglobin

Baseline %COHb from test₋₁ and test₋₂ did not differ (%COHb test₋₁ 0.87 versus test₋₂ 0.87 %, $p = 0.912$). In addition, %COHb measured at min-6 (5.8 % test₋₁ versus 5.8 % test₋₂, $p = 0.347$) and min-8 (5.7 % test₋₁ versus 5.7 % test₋₂, $p = 0.594$) post CO rebreathing did not differ between test₋₁ and test₋₂, see Figure 4-1 and Table 4-6 below.

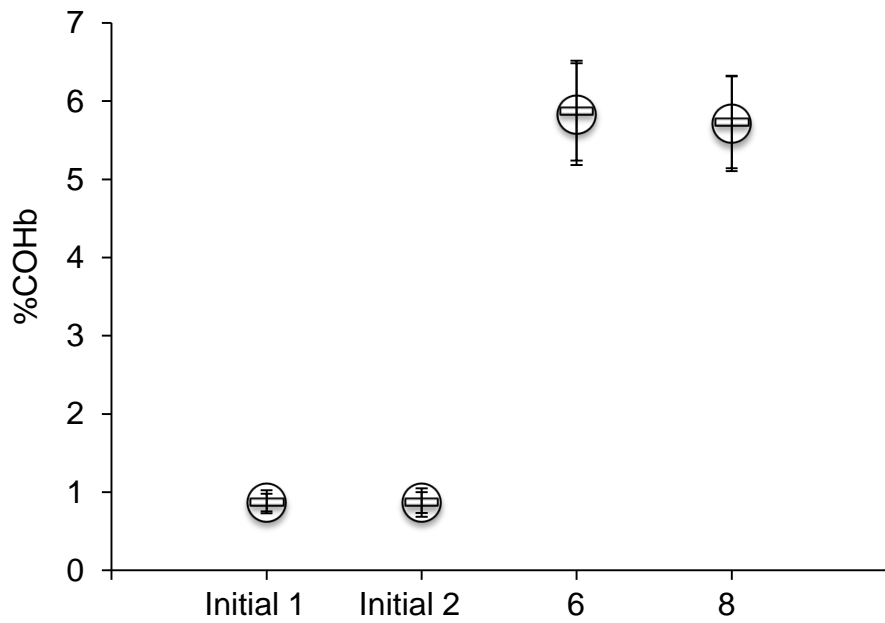


Figure 4-1. Percent carboxyhaemoglobin (%COHb) at baseline and at 6- and 8-min after CO rebreathing during oCOR test₁ (open circles) and oCOR test₂ (black rectangles). Error bars represent standard deviation (SD).

Table 4-3. Absolute total haemoglobin mass, red cell volume, blood volume and plasma volume from repeat optimised carbon monoxide rebreathing tests.

ID Number	tHb-mass (g)	tHb-mass (g)	RCV (ml)	RCV (ml)	BV (ml)	BV (ml)	PV (ml)	PV (ml)
	T1	T2	T1	T2	T1	T2	T1	T2
TRT01 (M)	733	730	2222	2078	5692	5566	3470	3487
TRT02 (M)	778	774	2401	2432	6230	6157	3829	3726
TRT03 (F)	448	452	1574	1478	4910	4667	3336	3189
TRT04 (F)	439	455	1491	1475	4183	4094	2691	2618
TRT05 (M)	908	906	2757	2434	6836	6399	4079	3965
TRT06 (F)	373	371	1251	1120	3610	3311	2359	2192
TRT07 (F)	535	532	1771	1696	5350	5197	3579	3502
TRT08 (F)	505	505	1516	1594	4323	4510	2807	2915
TRT09 (M)	737	735	2131	2360	5581	6046	3450	3686
TRT10 (M)	841	846	2600	2585	6222	6330	3622	3745
Mean	630	631	1971	1925	5294	5228	3322	3303
SD	190	188	521	514	1028	1055	539	568

oCOR, 2-minute optimised carbon monoxide rebreathing test; (M), male; (F), female; T1, test 1; T2, test 2; tHb-mass, total haemoglobin mass (grams, g), RCV, red cell volume (millilitres, ml); BV, blood volume (millilitres, ml), PV, plasma volume (millilitres, ml).

Table 4-4. Weight adjusted total haemoglobin mass, red cell volume, blood volume and plasma volume from repeat optimised carbon monoxide rebreathing tests.

ID Number	tHb-mass (g·kg ⁻¹) T1	tHb-mass (g·kg ⁻¹) T2	RCV (ml·kg ⁻¹) T1	RCV (ml·kg ⁻¹) T2	BV (ml·kg ⁻¹) T1	BV (ml·kg ⁻¹) T2	PV (ml·kg ⁻¹) T1	PV (ml·kg ⁻¹) T2
TRT01 (M)	11.3	11.2	34.2	32.0	87.6	85.6	53.4	53.6
TRT02 (M)	10.3	10.3	31.7	32.5	82.3	82.2	50.6	49.7
TRT03 (F)	6.8	6.7	23.9	22.1	74.6	69.7	50.7	47.6
TRT04 (F)	8.3	8.6	28.2	28.0	79.2	77.7	51.0	49.7
TRT05 (M)	10.2	10.2	30.8	27.3	76.5	71.9	45.6	44.6
TRT06 (F)	7.1	7.0	23.8	21.0	68.6	62.2	44.8	41.2
TRT07 (F)	8.3	8.3	27.5	26.3	83.1	80.7	55.6	54.4
TRT08 (F)	9.6	9.7	28.8	30.7	82.2	86.7	53.4	56.1
TRT09 (M)	10.6	10.6	30.8	34.1	80.6	87.4	49.9	53.3
TRT10 (M)	10.5	10.5	32.4	32.1	77.6	78.7	45.2	46.6
Mean	9.3	9.3	29.2	28.6	79.2	78.3	50.0	49.7
SD	1.6	1.6	3.5	4.5	5.3	8.2	3.7	4.8

oCOR, 2-minute optimised carbon monoxide rebreathing test; (M), male; (F), female; T1, test 1; T2, test 2; tHb-mass, total haemoglobin mass (grams per kilogram, g·kg⁻¹), RCV, red cell volume (millilitres per kilogram, ml·kg⁻¹); BV, blood volume (millilitres per kilogram, ml·kg⁻¹), PV, plasma volume (millilitres per kilogram, ml·kg⁻¹).

There were no differences in tHb-mass (g or g·kg⁻¹), RCV (ml or ml·kg⁻¹), BV (ml or ml·kg⁻¹), or PV (ml or ml·kg⁻¹) measured from repeat tHb-mass tests ($p > 0.05$). All repeat values were highly statistically significantly correlated [(tHb-mass, g & g·kg⁻¹ ($r = 1.000$, $p < 0.0001$; $r = 0.999$, $p < 0.0001$ respectively); RCV, ml & ml·kg⁻¹ ($r = 0.955$, $p < 0.0001$; $r = 0.888$, $p = 0.001$ respectively); BV, ml & ml·kg⁻¹ ($r = 0.964$, $p < 0.0001$; $r = 0.898$, $p = 0.001$ respectively); PV ml & ml·kg⁻¹ ($r = 0.966$, $p < 0.0001$; $r = 0.884$, $p = 0.002$ respectively)]. See preceding Table 4-3 and Table 4-4 for raw data in participants and Figure 4-3 showing the relationship between tHb-mass (g) measured from repeat oCOR tests.

4.4.4 Comparison of tHb-mass determined by the oCOR method

Figure 4-4 shows differences in tHb-mass between test-₁ and test-₂ in the Bland-Altman plot. The mean difference and SD in tHb-mass between test₁₋₂ was 1 ± 6 grams, with a very strong relationship between the two tHb-mass values ($r = 1.00$, $p < 0.0001$), see Figure 4-3. There were no differences in tHb-mass values from test-₁ and test-₂ (630 ± 190 g versus 631 ± 188 g, $p = 0.594$), see Table 4-5 (the same was the case for weight adjusted tHb-mass). The TE and 95 % confidence intervals (95 % CI) for tHb-mass values between test₁₋₂ for all patients ($n = 10$) was TE 1.9 % (95% CI 1.3-3.4 %). Based on the mean tHb-mass of 630 g from both tests, this would equate to a TE of 12 g (95% CI 8-22 g).

Table 4-5. Total haemoglobin mass for individual participants.

Participant	tHb-mass ₁	tHb-mass ₂	Mean tHb-mass ₁₋₂	Difference
1 (M)	733	730	732	3
2 (M)	778	774	776	4
3 (F)	448	452	450	-4
4* (F)	439	455	447	-17
5 (M)	908	906	907	2
6 (F)	373	371	372	2
7 (F)	535	532	534	3
8 (F)	505	505	505	0
9 (M)	737	735	736	1
10 (M)	841	846	844	-4
Mean	630	631	630	-1
SD	190	188	189	6

* tHb-mass value measured for participant 4 during test number 2 was affected by a major mouthpiece leak during CO rebreathing leading to overestimation of tHb-mass. Total haemoglobin mass, tHb-mass expressed in grams (g); (M), male; (F), female.

Table 4-6. Baseline percent carboxyhaemoglobin from test₁ for each participant.

Participant	%COHb ₁	%COHb ₂	Mean %COHb	Difference
1	0.9	0.8	0.85	0.1
2	0.8	0.9	0.85	-0.1
3	0.8	0.8	0.80	0.0
4	0.8	0.8	0.80	0.0
5	1.0	0.9	0.95	0.1
6	0.8	0.7	0.75	0.1
7	0.8	0.9	0.85	-0.1
8	0.7	0.7	0.70	0.0
9	1.0	1.1	1.05	-0.1
10	1.0	1.0	1.00	0.0
Mean	0.9	0.9	0.86	0.0
SD	0.11	0.13	0.11	0.08

%COHb, percent carboxyhaemoglobin. Data at baseline pre-CO rebreathing from oCOR test₁.

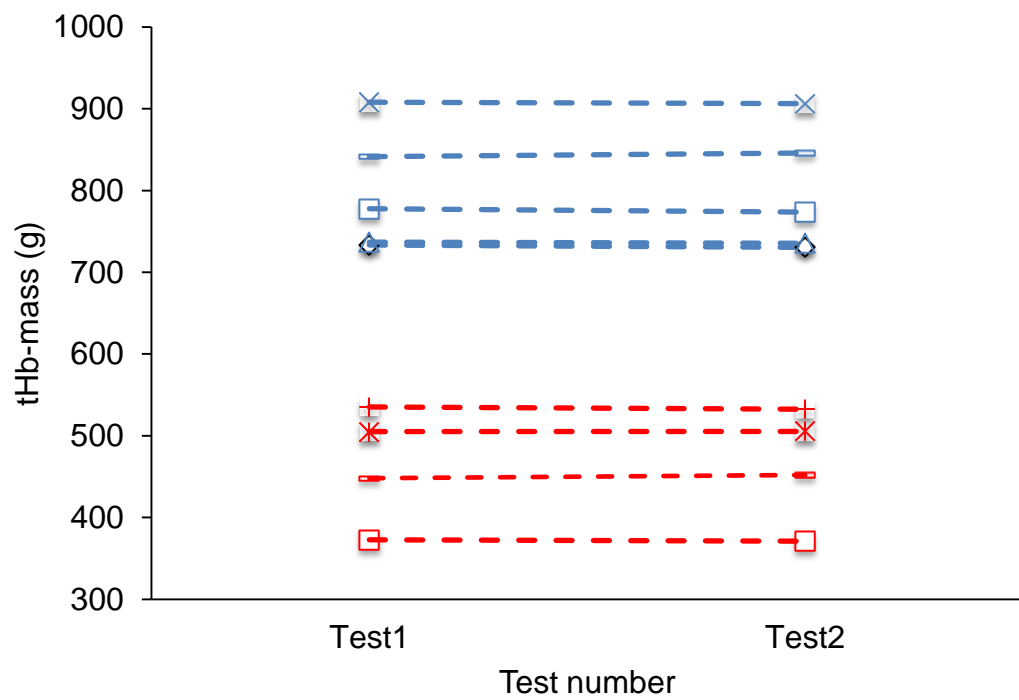


Figure 4-2. Individual total haemoglobin mass for each participant. Data expressed in grams (g). Red, females; blue, males.

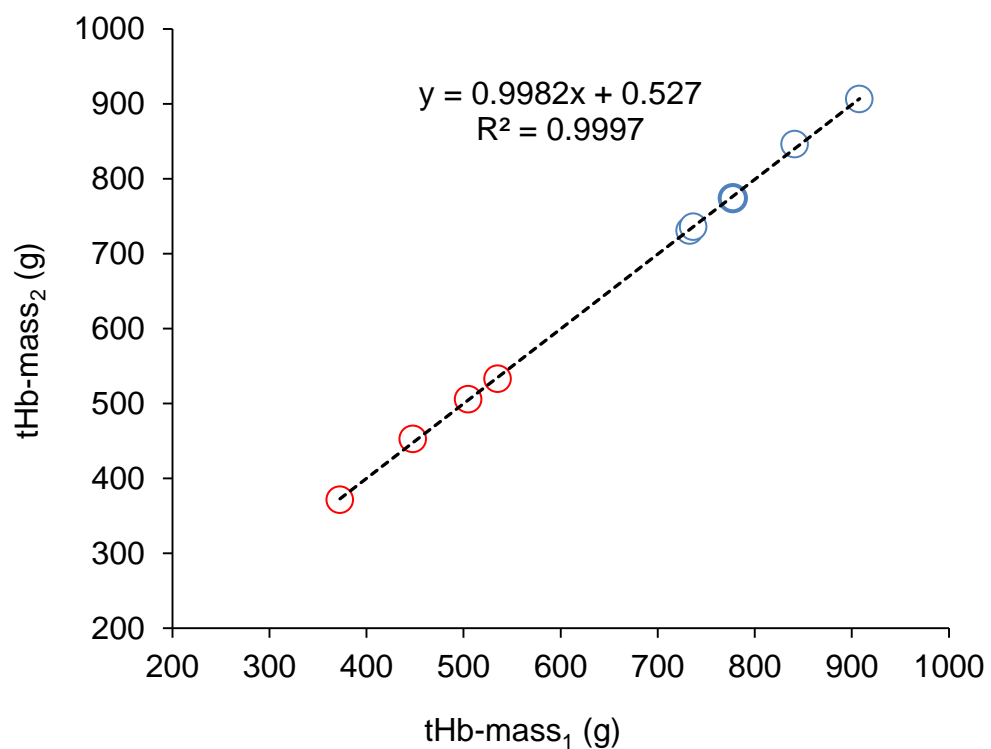


Figure 4-3. Correlation between total haemoglobin mass (tHb-mass) from test-1 (tHb-mass₁) and test-2 (tHb-mass₂). Data expressed in grams (g). Red, female; Blue, male.

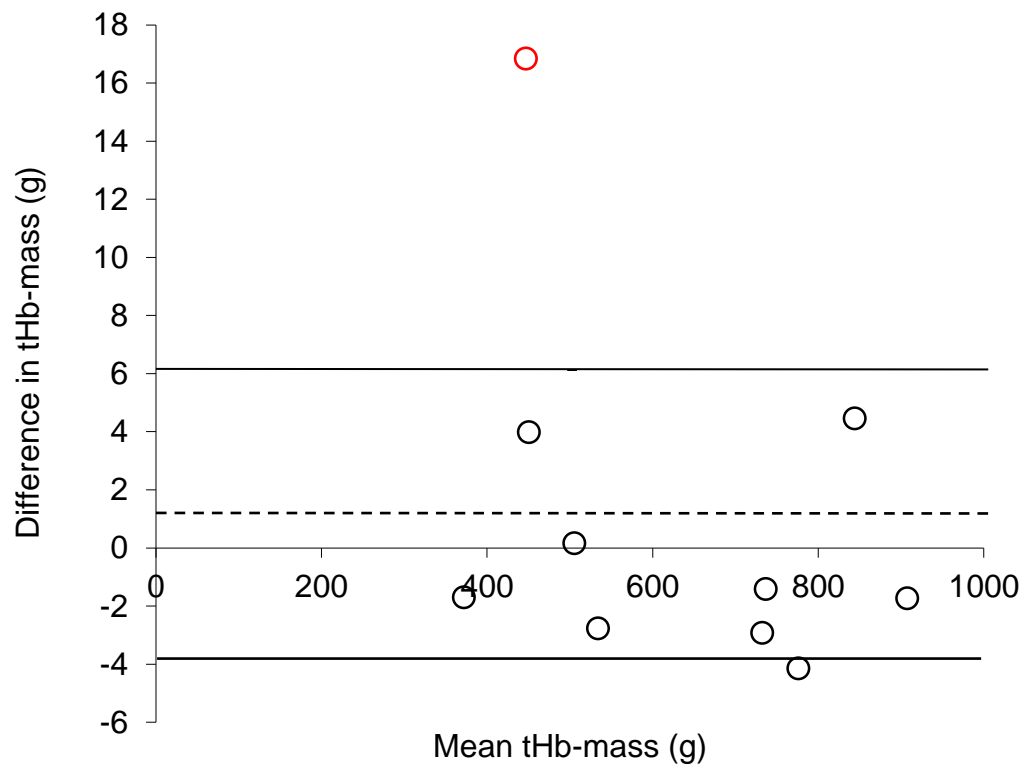


Figure 4-4. Bland-Altman plot for tHb-mass from test-1 and test-2 (expressed in grams of haemoglobin). The middle horizontal dashed line represents the overall mean difference (1 g) between test-1 and test-2 and the two solid lines represent the 95% limits of agreement (-4 to 6 g). Patient highlighted in red displayed evidence of a mouthpiece leak during testing but remains in the analysis for completeness.

4.5 Discussion

Test-retest reliability refers to the reproducibility of observed values when the measurement is repeated [290]. A test with better retest reliability indicates improved precision of single measurements and enhanced ability to track changes of measurements longitudinally over time [290]. Importantly any test cannot be valid (the ability of a measurement tool to reflect what it is designed to measure) unless it is reliable [291]. The main aim of this study was to quantify the test-retest measurement error (test-retest reliability) of total haemoglobin mass using the 2-minute optimised carbon monoxide rebreathing method in our testing institution at University College London Hospital. In addition, as tHb-mass determined by the oCOR method is dependent on the precision of %COHb measured in blood by a Hemoximeter [117], I assessed the repeatability of %COHb values obtained from the Cobas b 221 POC Hemoximeter used in this study.

The most important finding from this study was that the test-retest typical error of tHb-mass was 1.9 % or expressed in raw units was 9 grams of Hb, which demonstrates a level of reliability that is comparable to most previous studies (see Table 4-7 for a non-exhaustive list) that have utilised the 2-minute oCOR method. In addition, it is very similar to the 2.2% typical error associated with the oCOR method reported by Gore and colleagues [117], who performed a meta-analysis of errors of measurement in blood volume indices from the available literature. The original paper by Schmidt and Prommer in 2005 [19] (who developed, refined and patented the 2-minute oCOR method) reported a TE of 1.7 %. Subsequent studies have reported TE values ranging from 1.2 % [283] in 6 recreationally active adults to 3.3 % in national-level cyclists [27]. The pooled (average) TE % from all studies in Table 4-7 is 1.8%. The coefficient of repeatability (CR) of baseline %COHb derived from 5 capillary blood samples measured by the Cobas b 221 POC Hemoximeter was 0.14 %. This is low and is below the analyser error of $\leq 1\%$ essential for the accurate measurement of %COHb for subsequent tHb-mass calculation [284].

Table 4-7. Typical error of total haemoglobin mass measured using the 2-minute optimised carbon monoxide rebreathing method.

Authors	Sample size	Cohort	TE % (95% CI)
Schmidt and Prommer 2005 [19]	N= 11 (8 male)	Moderately trained adults	1.7 (3.3 %)
Gore et al. 2006 [283]	N= 6 (3 male)	Healthy, recreationally active adults	1.2 (0.8-2.5 %)
Garvican et al. 2010 [292]	N= 11 (all male)	6 male professional cyclists, 5 recreationally active controls	1.3 (0.9-2.5 %)
Eastwood et al. 2008 [282]	N= 6 (all male)	Active male adults	2.1 (1.7-2.6 %)
Eastwood et al. 2009 [293]	N= 23 (12 male)	Adolescents	2.3 (1.7-3.6 %)
Prommer et al. 2008 [44]	N= 24 (20 male)	Trained athletes	1.4 (1.1-1.7 %)
Schumacher et al. 2008 [27]	N= 7 (all male)	German national under-23 cyclists	3.3 % ^b
Pottgiesser et al. 2008 [46]	N= 29 (all male)	Healthy male adults	1.5 % ^{a b}
Clark et al. 2009 [36]	N= 12 (all male)	Endurance-trained cyclists	1.9 (1.5-3.0 %)
Robertson et al. 2010 [294]	N= 16 (11 male)	Highly trained runners	2.0 (1.6-2.6 %)
Naef et al. 2015 [295]	N= 18 (all male)	Healthy male adults	1.4 (1.0-2.1 %) ^c

All participants performed the 2-minute optimised carbon monoxide rebreathing method [19] to determine total haemoglobin mass (tHb-mass). TE % (95% CI), Typical error (95% confidence interval). ^aTE derived from 11 participants, ^b95% CI not provided, ^ctHb-mass measured twice within 6 hours. Average TE for all studies is 1.8%.

The data obtained from the current study on the reliability of tHb-mass allows the quantification of the precision of a single, one-off measurement of tHb-mass in an individual. Specifically, the observed within-subject typical error was 1.9 % (95% CI 1.3-3.4 %). Based on the mean tHb-mass of 630 grams (average tHb-mass from test₁ and test₂), and calculated TE (1.9 %), the 95% likely limits for this single tHb-mass measurement is between 604 to 657 grams (a range of 54 grams). In other words, we can be confident that in our hands, the probability of a one-off measurement of tHb-mass (g) falling outside these limits is 5%. Conversely, the probability of a one-off measurement of tHb-mass falling within this range is 95%.

The calculation of these likelihood limits can be used to calculate the probability that the 'true' tHb-mass for a subject or patient is greater (or less) than some predefined value or a value with clinical importance/significance. This has important implications for interpreting data in future studies to qualify whether a 'true', real-world change in tHb-mass has occurred. In other words, is the change in tHb-mass greater than the error associated with the method of measurement. If not, then any change could simply be a consequence of measurement error and our interpretation of results may be limited. This underlying principle applies to all measurement techniques in sports and clinical medicine, although is perhaps overlooked.

What criteria or threshold can we use when deciding whether a real-world change in tHb-mass has occurred? Hopkins [290] suggests a realistic approach is to base such criteria on the TE of the test or method being used. Specifically, 1.5 to 2 times the typical error (i.e. greater than half the limits of agreement) has been advocated. Accordingly, based on the mean tHb-mass (g) from the current study (630 g), limits of agreement (-4 to 6 g) and typical error of 1.9 % (equates to 9 g in raw units), the oCOR rebreathing method in our institution has the precision to detect a real-world change in tHb-mass of ≥ 14 g (1.5 x TE) with sufficient confidence. If we use 2 times the TE, this equates to a change in tHb-mass of 18 g.

In real terms this equates to an ability to detect a change in tHb-mass associated with reinfusion or withdrawal of one unit of packed red cells (containing ~ 60 g of Hb) [296] or changes in tHb-mass in the early phase of high-dose recombinant human erythropoietin administration, as shown by Parisotto and colleagues, where a mean increase in tHb-mass of between 58-107 g was reported [262]. However, the oCOR rebreathing method may not have the ability to detect changes in tHb-mass during periods of micro-dosing or 'maintenance' with recombinant human erythropoietin administration [297]. This may be related to such changes being close to the natural oscillations in tHb-mass that have been reported (4.6% average oscillation in tHb-mass recorded over a one year period in endurance-trained athletes [298]. The stability of tHb-mass in clinical populations has been little studied and what would be considered a normal oscillation in tHb-mass in this context remains to be explored in different patient and disease populations.

4.5.1 Study limitations

The small sample size in this study was a potential limitation, although 10 participants is in keeping with 6 of the studies outlined in Table 4-7 that have utilised the 2-minute oCOR method to determine tHb-mass and reported TE values that are similar to that reported in the current study (i.e. ~2 %). Furthermore, as Hopkins suggests [290], when the typical error has the same or very similar magnitude as the smallest worthwhile effect, a sample size of 10 provides sufficient precision in a simple test-retest study without a control group. This is based on Equation 4-1 below.

Equation 4-1. Sample size formula based on typical error and smallest worthwhile effect.

$$n = 2(t \times s/d)^2 \approx 8s^2/d^2$$

Where n is the estimated sample size; t is the test statistic; s is the typical error and d is the smallest worthwhile effect.

If we use the calculated typical error for tHb-mass of 1.9 % derived from the current study and an assumed smallest worthwhile effect of 1.8 % (pooled TE from studies reported in Table 4-7, a sample of 9 participants gives adequate precision in a simple test retest experiment. Given that sample size is proportional to the square of the typical error, a high level of reliability is extremely important in any research experiment [290]. In Chapter 4 I have shown a low TE in the repeat measurement of tHb-mass using the oCOR method in our institution.

4.6 Conclusion

This study demonstrates that tHb-mass measured using the 2-minute optimised carbon monoxide rebreathing method is of sufficient reliability in our testing institution compared to previous studies using this technique. In addition, the analyser error of the Cobas b 221 POC Hemoximeter was low and less than that essential ($\leq 1\%$) for the accurate measurement of %COHb for subsequent calculation of tHb-mass. Finally, the measurement error in tHb-mass using the oCOR method suggests that clinically important changes in tHb-mass can be detected following targeted intervention, such as for example following infusion of packed RBCs, use of rhEPO and intravenous iron supplementation.

Chapter 5

- 5 Haemoglobin concentration, total haemoglobin mass and plasma volume in patients: implications for anaemia**

5.1 Abstract

Background. Anaemia is a common haematological disorder encountered in many clinical settings and is defined when [Hb] is $< 120 \text{ g}\cdot\text{l}^{-1}$ in females and $< 130 \text{ g}\cdot\text{l}^{-1}$ in males, according to World Health Organisation criteria. Because [Hb] is based on whole blood, it is dependent on both PV and the total amount of circulating haemoglobin (tHb-mass), which may both affect [Hb] independently of one another, but are rarely independently measured in clinical medicine. Thus, anaemia may result not only from a fall in tHb-mass, but from a simple rise in PV. Therefore, reliance on [Hb] values may be misleading. We sought to explore the relationship between tHb-mass and [Hb] in healthy volunteers and in different exemplar patient populations to test the hypothesis that tHb-mass is poorly correlated with [Hb].

Methods. Healthy volunteers (HV) and patients from the following disease groups were studied; chronic liver disease (CLD), chronic heart failure (CHF), inflammatory bowel disease (IBD) and those awaiting elective surgery (surgical). tHb-mass was quantified using the optimised carbon-monoxide (oCOR) rebreathing method and circulating [Hb] determined.

Results. One hundred and nine participants (61% male), mean (IQR) age 52 (36-64) years were enrolled and successfully completed the oCOR test, of which 36% were anaemic. Mean \pm SD [Hb] and tHb-mass (g & $\text{g}\cdot\text{kg}^{-1}$) were $128.4 \pm 18.1 \text{ g}\cdot\text{l}^{-1}$, $669 \pm 181 \text{ g}$ and $8.5 \pm 1.9 \text{ g}\cdot\text{kg}^{-1}$, respectively. There was no difference in tHb-mass (g or $\text{g}\cdot\text{kg}^{-1}$) between sub-groups, although [Hb] and Hct (%) were significantly lower in CLD patients compared to all other groups, owing to expanded plasma volume. In all participants tHb-mass was correlated with [Hb] ($r = 0.50$, $p < 0.0001$) but this relationship varied across sub-groups (HV $r = 0.87$, $p < 0.0001$; surgical $r = 0.787$, $p < 0.0001$; IBD $r = 0.687$, $p < 0.0001$), although tHb-mass and [Hb] were not correlated in CLD ($r = 0.410$, $p = 0.114$) or CHF ($r = 0.312$, $p = 0.157$). tHb-mass did not explain variance in [Hb] in CLD (adjusted $R^2 = 0.109$, $p = 0.114$) or CHF (adjusted $R^2 = 0.052$, $p = 0.157$) with PV independently accounting for a greater proportion of the variance in [Hb] over and above tHb-mass in these group (R^2 change = 0.724 in CHF and 0.805 in CLD).

Conclusions. The relationship between tHb-mass and [Hb] varies considerably in different disease states with plasma volume a key confounding variable

showing wide variability on an individual patient level. Knowledge of tHb-mass, PV and [Hb] provides a more comprehensive assessment of haematological and volume status in patients compared to relying on [Hb]. This study raises the question as to whether the concept of 'anaemia' needs readdressing and if we should be defining volume excess and tHb-mass deficit separately instead?

5.2 Introduction

Anaemia is a common haematological disorder encountered in many clinical settings and disease states such as cancer [299], IBD [7], CHF [300], chronic kidney disease [301, 302], chronic liver disease (LD) [8] and in patients preoperatively [303, 304]. Anaemia regardless of aetiology is associated with reduced quality of life [305], chronic fatigue [306, 307] and poor prognosis across a range of diseases from CHF [308, 309] to various malignancies [15] as well as poor postoperative outcomes in cardiac [181, 182] and non-cardiac [6, 13] surgery. For all these reasons, circulating haemoglobin concentrations ([Hb]) are routinely measured in clinical practice. Once identified, the cause of impaired haemoglobin synthesis or erythrocytosis, or of increased red cell destruction, is often sought and treatment (either of the underlying cause, or through the administration of packed red cells) instigated.

It is becoming clear that our understanding of the pathogenesis of anaemia, and thus of investigations and treatment responses, may have been somewhat simplistic. Traditionally anaemia has been considered the result of a reduced tHb-mass (impaired O₂ carriage) but also, given that measured [Hb] is based on whole blood, it is dependent on PV, which may affect [Hb] independently- a factor rarely considered in clinical practice. But a low [Hb] might also be found when haemoglobin synthesis and erythrocytosis are normal, and when tHb-mass is normal (or even high), if plasma volume is disproportionately expanded. For example, disease-related changes in global water balance or distribution of body water or the use of diuretic therapy may disturb PV. Thus, patients with IBD might face intestinal blood loss, suppressed haemoglobin synthesis (anaemia of chronic inflammation) or hypoalbuminaemia, causing fluid shifts relating to altered plasma oncotic pressure [310]. In CLD, patients may

similarly experience blood loss and fluid shifts as a result of raised portal venous pressure [99, 311], hypoalbuminaemia and the use of diuretic therapy [312]. Patients with CHF may suffer disturbances in circulating blood volumes due to raised central venous pressure, increased renin-angiotensin-aldosterone activity, and the use of diuretics [93, 95]. Indeed, PV expansion resulting in haemodilution is a recognised feature of HF [96, 97]. In contrast, contractions in PV caused by pharmacotherapy may mask a fall in tHb-mass by maintaining [Hb] [98].

Why may this be of clinical relevance? [Hb] is used as a trigger for further investigations and treatment in a number of settings from anaemia management to transfusion requirements in surgery. If a reduced [Hb] is not a result of a 'true' deficit in tHb-mass, is a patient's O₂ carrying capacity impaired and is Hb optimisation necessary? In contrast, a patient may have a reduced [Hb] as a result of hypervolaemia (i.e. PV expansion) without any deficit in tHb-mass. The clinical management of these two scenarios may be vastly different. For example, in a patient with CHF, optimisation of Hb may be required in the first scenario whereas appropriate management of volume status may be appropriate in the second. Therefore, knowledge of tHb-mass, PV and [Hb] may provide a more comprehensive assessment of haematological and volume status compared to relying on [Hb] alone. Furthermore do we need to readdress 'anaemia' as a concept and should we be defining volume excess and tHb-mass deficit separately?

Previous techniques used to quantify red cell mass (actually a measurement of red cell volume, ml) have involved radiolabelling of red blood cells with chromium (⁵¹Cr) and are therefore tools limited to nuclear medicine departments. The use of isotopes for the measurement of blood volume compartments are not without associated risks to the patient, are costly and place a burden on the patient in terms of the time and procedures required to perform, and are thus not routinely performed unless for special circumstances (such as the management of excessive erythrocytosis in polycythaemia). These barriers may have limited the applicability of such techniques for more routine and regular use in clinical medicine. In contrast, such barriers may be overcome through use of the 2-minute oCOR method, refined by Schmidt and

Prommer [19], which offers a less invasive, safe, precise and reliable technique to measure tHb-mass. In addition, the 2-minute oCOR method has comparable if not lower measurement error when compared to previous blood volume techniques. Despite these advantages it has rarely been applied in the clinical setting. Thus, the relative contributions of PV and tHb-mass to measured [Hb] across disease states has not been well described. I sought to address this issue, studying patients suffering IBD, CLD and CHF, I also studied healthy volunteers (HV), as well as a preoperative patient cohort.

5.2.1 Study aim

- i) To explore the relationship between total haemoglobin mass and haemoglobin concentration in different disease states.
- ii) To assess the feasibility of using the optimised carbon monoxide rebreathing method in patients.

5.2.2 Study hypothesis

Total haemoglobin mass will be poorly correlated with haemoglobin concentration.

5.3 Methods

Ethical approval was granted by the London- Camden and Kings Cross Research Ethics Committee (REC reference: 13/LO/1902). We fully adhered to Caldicott guidelines and conformed to the standards set by the Declaration of Helsinki. Patients were given a participant information sheet (see Appendix C) at least 24 hours prior to written informed consent being obtained from all participants willing to take part (see Appendix D).

5.3.1 Patients

Adult (> 18 years) healthy volunteers and patients at University College Hospital (University College London Hospitals NHS Foundation Trust, UCLH), Southampton General Hospital (University Hospital Southampton NHS Foundation Trust) and the Royal Free Hospital (Royal Free London NHS Foundation Trust, RFH) were prospectively studied between February 2015 and May 2016. Age, gender, height, weight, diagnosis, planned surgical procedure, comorbidities (such as diagnosis of diabetes, respiratory or cardiovascular disease) and current medications were also documented.

5.3.2 Inclusion criteria

5.3.2.1 Healthy volunteers (HV)

- i) Male or female adults (age > 18 years)
- ii) Free from chronic disease

5.3.2.2 Chronic heart failure (CHF)

- i) Heart failure confirmed with B-type natriuretic peptide (BNP) > 200 pg·ml⁻¹ (if available)
- ii) Echocardiogram or cardiac magnetic resonance imaging (cMRI)

evidence of left ventricular (LV) impairment as defined by a left ventricular ejection fraction of less than 50%

- iii) New York Heart Association (NYHA) class II-IV
- iv) Not fully stabilised heart failure, i.e. still symptomatic with shortness of breath and/or peripheral oedema and/or clinical signs of bibasal crepitations or peripheral oedema

5.3.2.3 Chronic liver disease of mixed aetiology (CLD)

- i) Alcoholic liver disease
- ii) Primary sclerosing cholangitis
- iii) Hepatitis C related liver cirrhosis
- iv) Cryptogenic cirrhosis

5.3.2.4 Gastrointestinal disease

- i) Confirmed diagnosis of Inflammatory bowel disease (IBD)
 - a. Crohn's disease
 - b. Ulcerative colitis

5.3.2.5 Surgical patients (Surgical)

- i) Patients scheduled to undergo elective surgery regardless of specialty

5.3.3 Exclusion criteria

- i) Adults with known underlying history of learning disabilities, or adults who do not have mental capacity to consent for themselves.
- ii) Prisoners
- iii) Unable to comply with basic breath-holding instructions.
- iv) Baseline %COHb is greater than 5% (as commonly occurs in smokers).
- v) Haemoglobinopathies (e.g. Sickle cell anaemia).

5.3.4 Statistics

Statistical analysis was performed using SPSS Statistics (Version 23.0 for Apple Macintosh, Chicago, IL, USA). Values are presented as mean \pm standard deviation (SD), unless otherwise stated. Median and interquartile range (IQR) are reported when variables are not normally distributed and when data transformation (logarithmic, square root or reciprocal) did not result in a normally distributed variable. Categorical variables are presented as frequency (%). Normal (Gaussian) distribution was assessed using a combination of the Kolmogorov-Smirnov test, visual inspection of histogram charts and normal Q-Q plots for each variable. Pearson's correlation coefficient assessed the relationship between [Hb] and tHb-mass, allowing adjustment for PV (ml). Linear regression assessed the proportion of variance in tHb-mass explained by [Hb], allowing adjustment for PV (ml). In both correlation and regression analyses [Hb] and tHb-mass are expressed in g·l⁻¹ and grams, respectively and PV in ml, unless otherwise stated. This is also the case elsewhere, unless otherwise stated.

Differences across sub-groups were assessed by one-way analysis of variance (ANOVA), prior to which the assumption of normality was tested by the Levene's test for homogeneity of variances. Where homogeneity of variance was verified, being the case for [Hb], Hct, tHb-mass (g), RCV (ml & ml·kg⁻¹), PV (%) and weight, a one-way ANOVA, with *post hoc* comparisons by Gabriel's

test (due to the slightly different sample sizes across sub-groups) was performed. When homogeneity of variance was violated, as was the case for BV (ml & ml·kg⁻¹), PV (ml & ml·kg⁻¹), age and tHb-mass (g·kg⁻¹), a Welch ANOVA [313] was performed with subsequent *post hoc* comparisons made by the Games-Howell test as this post hoc test does not rely on the assumption of equal variances [314]. All tests were two-sided with statistical significance being accepted as a p-value of < 0.05.

5.3.5 Equations

Body surface area (BSA) was derived from height (H) and body weight (W) using the formula of Du Bois and Du Bois [316]:

Equation 5-1. Du Bois and Du Bois body surface area formula

$$BSA[m^2] = 0.007184 \times H[cm]^{0.725} \times W[kg]^{0.425}$$

Estimation of normal adult values for RCV and PV were calculated using the formulas from the 1995 Expert Panel on Radionuclides of the International Council for Standardisation in Haematology [92]:

Equation 5-2. Estimation of normal male adult red cell volume and plasma volume

$$\text{Mean normal RCV (ml)} = (1486 \times S) - 825$$

$$\text{Mean normal PV (ml)} = 1578 \times S$$

Equation 5-3. Estimation of normal female adult red cell volume and plasma volume

$$\text{Mean normal RCV (ml)} = (1.06 \times \text{age}) + (822 \times S)$$

$$\text{Mean normal PV (ml)} = 1395 \times S$$

where S = body surface area (m^2) calculated using the formula of Du Bois and Du Bois [316] (see equation 5-1 above).

Equation 5-4. Calculation of mean normal blood volume

$$\text{Mean normal BV (ml)} = \text{mean normal RCV} + \text{mean normal PV}$$

BV and PV values were adjusted for age, sex, weight, and height using a published formula to calculate normal volumes as derived from >100,000 measurements of height and weight from Metropolitan Life tables [91]. Normal BV, PV and RCV were classified as measured volumes within $\pm 8\%$ of the expected normal volume on an individual level. Mild to moderate volume expansion was considered $>8\%$ to $<25\%$ deviation from expected norms, and severe as $>25\%$ of the expected normal volume [91].

5.3.6 Power calculation

The power calculation was based on available literature, specifically using the study by Hinrichs and colleagues [24] and was performed using G*3 Power version 3.1.9.2 [316]. According to Hinrichs and colleagues, the relationship (expressed as Pearson's correlation coefficient) between haemoglobin concentration ([Hb]) and total haemoglobin mass (tHb-mass) was $r = 0.59$, $p < 0.05$. Based on this, the number of patients using a two-tailed correlation: bivariate normal model, for 80% power with a significance of 0.05 is estimated at 21 per group. Given that five groups were studied (patient populations; IBD, surgical, liver disease and chronic heart failure) and healthy volunteers, a total of 105 participants were required.

5.4 Results

One hundred and nine participants (61 % male), mean (IQR) age 52 (36-64) years consented to take part in the study. Sixteen patient were tested at Southampton General Hospital, ninety at University College London Hospital (inclusive of all healthy volunteers) and three at the Royal Free Hospital. Surgical specialities of planned procedures are shown in Table 5-1 with patient characteristics and aetiology of disease of inflammatory bowel disease patients in Table 5-2. Patient characteristics, medications and aetiology of liver disease and chronic heart failure are shown in Table 5-3 and Table 5-4, respectively. There were no differences in weight between sub-groups. HV were younger compared to all patient groups ($p < 0.0001$) with CHF patients being older than IBD ($p = 0.001$), surgical ($p = 0.008$) and LD patients ($p = 0.002$). In the whole group 36% were anaemic according to WHO criteria ($< 120 \text{ g}\cdot\text{l}^{-1}$ in females and $< 130 \text{ g}\cdot\text{l}^{-1}$ in males). The prevalence of anaemia in sub-groups were as follow: IBD (9%), HV (24%), surgical (18%), HF (55%) and LD (94%).

Table 5-5 shows haematological variables in male and female participants. Males displayed elevated [Hb], tHb-mass (g , $\text{g}\cdot\text{kg}^{-1}$ & $\text{g}\cdot\text{m}^2$) as well as BV and PV (ml & $\text{ml}\cdot\text{m}^2$) but not when adjusted for measured body weight. RCV (ml & $\text{ml}\cdot\text{kg}^{-1}$ & $\text{ml}\cdot\text{m}^2$) were also higher in males compared to females. Table 5-6 shows haematological variables in anaemic and non-anaemic participants. Anaemic subjects displayed lower [Hb], Hct (%), RCV (ml & $\text{ml}\cdot\text{kg}^{-1}$ & $\text{ml}\cdot\text{m}^2$) and tHb-mass (g , $\text{g}\cdot\text{kg}^{-1}$ & $\text{g}\cdot\text{m}^2$) compared to non-anaemics. In contrast, BV ($\text{ml}\cdot\text{kg}^{-1}$ only) and PV (ml , $\text{ml}\cdot\text{kg}^{-1}$, $\text{ml}\cdot\text{m}^2$ & %) were higher in anaemic participants. Also, participants classified as anaemic displayed reduced MCV and elevated RDW compared to non-anaemic subjects.

Table 5-7 shows haematological variables for the whole study cohort, healthy volunteers and in different disease sub-groups. These values for Hct, [Hb] and tHb-mass are also displayed graphically in Figure 5-1 (A-D) and for BV and PV in Figure 5-2 (A-D), respectively. tHb-mass, RCV, BV and PV expressed relative to BSA (m^2) are expressed in Figure 5-3 (A-D). LD patients had lower [Hb] and Hct compared to healthy volunteers and other disease groups ($p < 0.0001$). tHb-mass (g , $\text{g}\cdot\text{kg}^{-1}$ & $\text{g}\cdot\text{m}^2$) did not differ between sub-groups nor did

RCV (ml). However, RCV ($\text{ml}\cdot\text{kg}^{-1}$) in LD patients was lower than HV ($p= 0.006$) and HF ($p= 0.006$) and when expressed relative to BSA, RCV ($\text{ml}\cdot\text{m}^2$) was lower in LD compared to patients with HF ($p= 0.047$).

BV (ml) was expanded in LD patients compared to IBD ($p= 0.038$), surgical patients ($p= 0.037$) but was not different from CHF patients or HV. Weight adjusted BV ($\text{ml}\cdot\text{kg}^{-1}$) was greater in HF ($p= 0.016$) and HV ($p= 0.008$) compared to surgical patients. BV ($\text{ml}\cdot\text{m}^2$) was higher in LD compared to surgical patients ($p= 0.035$), which was also the case for BV ($\text{ml}\cdot\text{m}^2$) in HF patients compared to IBD ($p= 0.032$) and surgical ($p= 0.014$). In HV, BV ($\text{ml}\cdot\text{m}^2$) was higher compared to surgical patients ($p= 0.046$).

PV (ml) was expanded in LD compared to HV ($p= 0.006$), IBD and surgical patients ($p= 0.002$) but not patients with HF ($p= 0.100$). PV expressed relative to body weight ($\text{ml}\cdot\text{kg}^{-1}$) was expanded in LD patients compared to IBD ($p= 0.007$) and surgical patients ($p= 0.003$) but not HF ($p= 0.172$) or HV ($p= 0.191$). HV demonstrated a larger PV ($\text{ml}\cdot\text{kg}^{-1}$) compared to IBD and surgical patients, respectively. PV ($\text{ml}\cdot\text{m}^2$) expressed relative to BSA was expanded in LD patients compared to IBD ($p= 0.001$), surgical ($p= 0.001$) and HV ($p= 0.015$) but not HF ($p= 0.080$). In HF patients PV ($\text{ml}\cdot\text{m}^2$) was elevated compared to IBD ($p= 0.014$) and surgical ($p= 0.008$). Finally, HV displayed higher PV ($\text{ml}\cdot\text{m}^2$) compared to both IBD ($p= 0.036$) and surgical patients ($p= 0.014$).

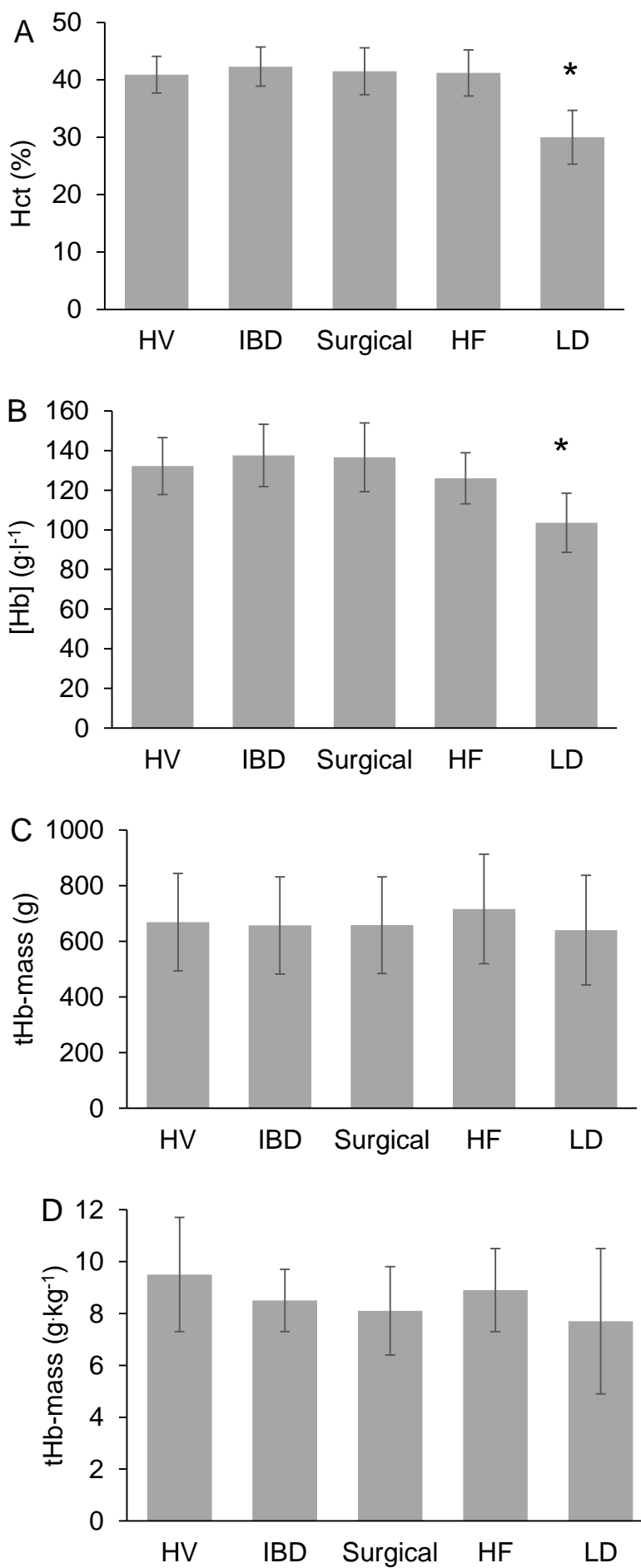


Figure 5-1 (A-D)

Figure 5-1. Haematological variables in healthy volunteers and patient sub-groups. Hct (%), haematocrit percentage (A); [Hb], haemoglobin concentration (B); tHb-mass, total haemoglobin mass (g & g·kg⁻¹) (C & D). HV, healthy volunteers; IBD, inflammatory bowel disease; HF, heart failure; LD, liver disease. *p< 0.0001 for Hct and [Hb] in LD patients compared to all other groups. No differences in tHb-mass (g or g·kg⁻¹) between groups.

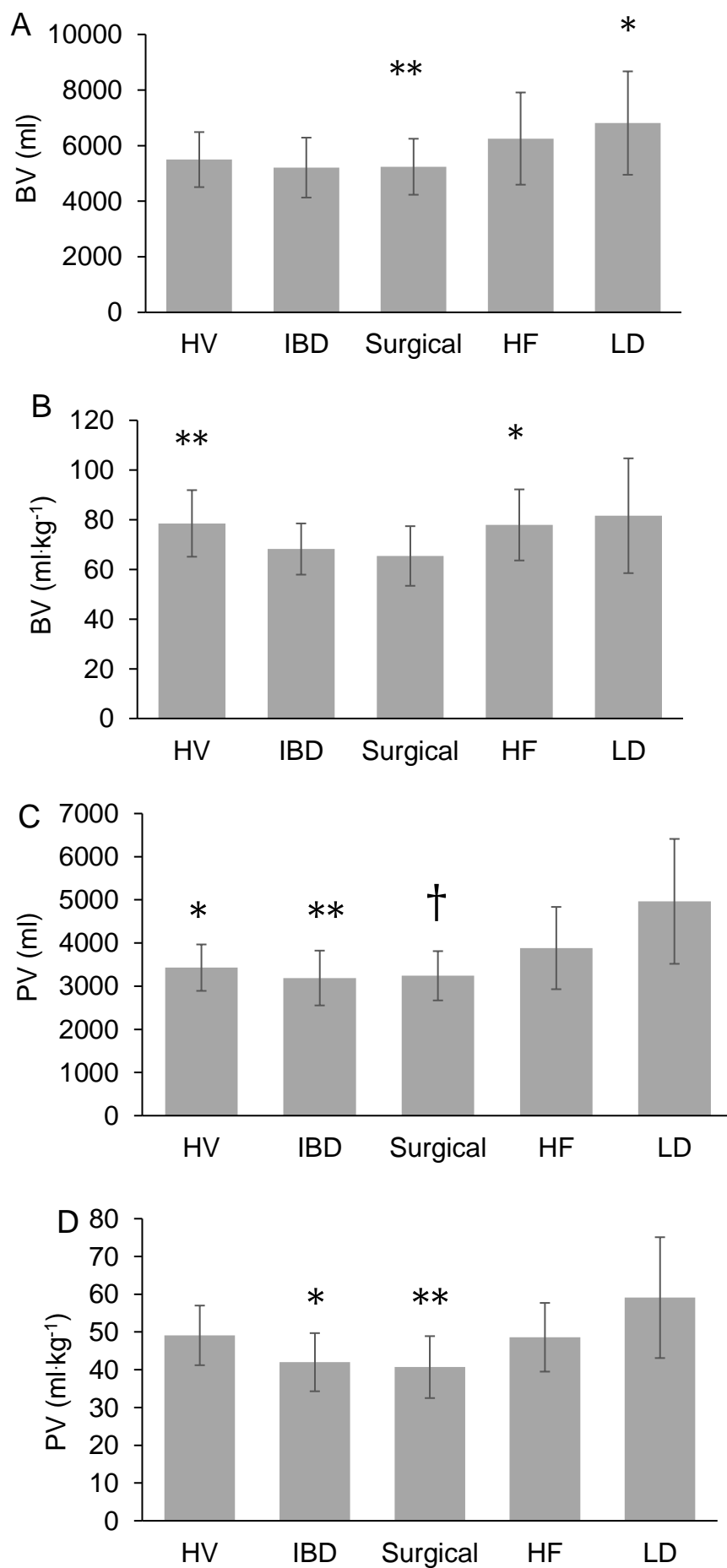


Figure 5-2 (A-D)

Figure 5-2. Blood and plasma volumes in healthy volunteers and patient sub-groups. BV, blood volume (ml & ml·kg⁻¹) (A & B); PV, plasma volume (ml & ml·kg⁻¹) (C & D). HV, healthy volunteers; IBD, inflammatory bowel disease; HF, heart failure; LD, liver disease. (A) BV (ml) between LD and IBD, *p= 0.038, BV (ml) between LD and surgical, **p= 0.037; (B) BV (ml·kg⁻¹) between HF and surgical, *p= 0.016, BV (ml·kg⁻¹) between HV and surgical, **p= 0.008; (C) PV (ml) between LD and HV, *p= 0.006, PV (ml) between LD and IBD, **p= 0.002 and PV (ml) between LD and surgical patients, †p= 0.002; (D) PV (ml·kg⁻¹) between LD and IBD, *p= 0.007, PV between LD and surgical patients, **p= 0.003.

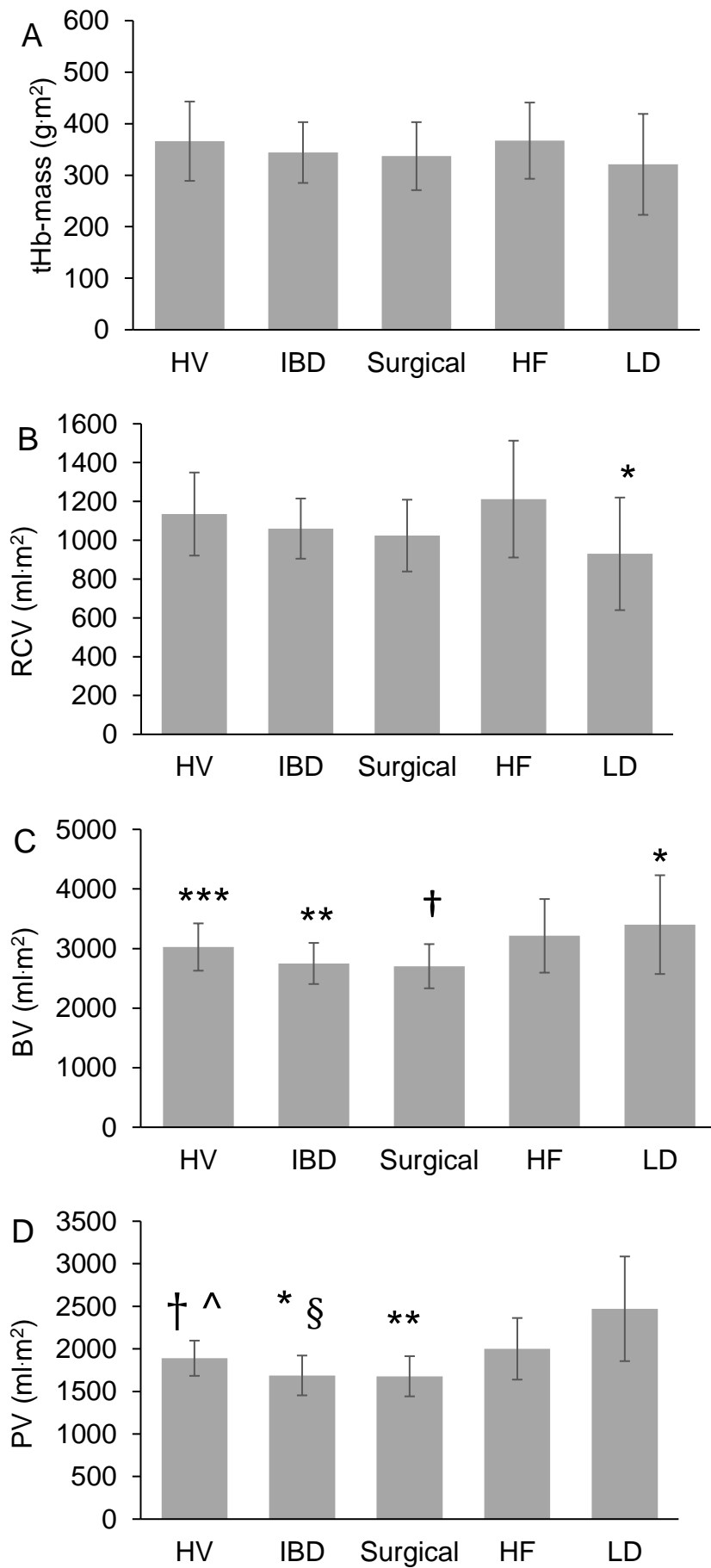


Figure 5-3 (A-D)

Figure 5-3. Total haemoglobin mass, blood, plasma and red cell volumes expressed relative to body surface area (m^2) in healthy volunteers and patient sub-groups. tHb-mass, total haemoglobin mass ($\text{g}\cdot\text{m}^2$) (A); RCV, red cell volume ($\text{ml}\cdot\text{m}^2$) (B); BV, blood volume ($\text{ml}\cdot\text{m}^2$) (C); PV, plasma volume ($\text{ml}\cdot\text{m}^2$) (D). HV, healthy volunteers; IBD, inflammatory bowel disease; HF, heart failure; LD, liver disease. No difference in tHb-mass ($\text{g}\cdot\text{m}^2$). (B) RCV ($\text{ml}\cdot\text{m}^2$) lower in LD than HF, $^*p=0.047$; (C) BV ($\text{ml}\cdot\text{m}^2$) in LD higher than surgical, $^*p=0.035$, BV ($\text{ml}\cdot\text{m}^2$) in HF higher than IBD, $^{**}p=0.032$ and surgical, $^{\dagger}p=0.014$, $^{***}p=0.001$, BV ($\text{ml}\cdot\text{m}^2$) in HV higher than surgical ($p=0.046$); (D) PV ($\text{ml}\cdot\text{m}^2$) higher in LD compared to IBD, $^*p=0.001$, surgical, $^{**}p=0.001$ and HV $^{\dagger}p=0.015$ but not HF, $p=0.080$, PV ($\text{ml}\cdot\text{m}^2$) higher in HV compared to IBD, $^{\S}p=0.036$ and surgical, $^{\wedge}p=0.014$.

5.4.1 Relationships between haemoglobin concentration and absolute total haemoglobin mass

In the whole population tHb-mass (g) correlated with [Hb] ($\text{g}\cdot\text{l}^{-1}$) ($r = 0.500$, $p < 0.0001$, $n = 109$), which varied slightly by gender [(males ($n = 66$) $r = 0.452$, $p < 0.0001$; female ($n = 43$) $r = 0.462$, $p < 0.0001$)]. In HV, IBD and surgical patients tHb-mass was strongly related with [Hb] (HV $r = 0.871$, $p < 0.0001$; IBD $r = 0.687$, $p < 0.0001$; surgical $r = 0.787$, $p < 0.0001$), see Figure 5-9. However, this was not the case in patients with LD or CHF (LD $r = 0.410$, $p = 0.114$; CHF $r = 0.312$, $p = 0.157$). After statistically adjusting for PV (ml), tHb-mass was strongly related to [Hb] in all groups (HV $r = 0.965$, $p < 0.0001$; IBD $r = 0.976$, $p < 0.0001$; surgical $r = 0.985$, $p < 0.0001$; LD $r = 0.984$, $p < 0.0001$; CHF $r = 0.905$, $p < 0.0001$).

In all anaemic patients ($n = 39$), tHb-mass did not significantly correlate with [Hb] ($r = 0.301$, $p = 0.062$) but did to a moderate extent in all patients without anaemia ($r = 0.562$, $p < 0.0001$). Where analysis was possible due to very small sample sizes after patients were split by sub-group and anaemia status, tHb-mass was not significantly related to [Hb] in anaemic patients [(HV ($n = 5$) $r = 0.204$, $p = 0.742$; surgical ($n = 5$) $r = 0.669$, $p = 0.217$; HF ($n = 12$) $r = 0.462$, $p = 0.131$; LD ($n = 15$) $r = 0.338$, $p = 0.218$; IBD ($n = 2$)]. In non-anaemic participants ($n = 70$), tHb-mass was significantly correlated with [Hb] in HV ($n = 16$), $r = 0.839$, $p < 0.0001$; surgical ($n = 23$) $r = 0.652$, $p = 0.001$ and IBD ($n = 20$) $r = 0.644$, $p = 0.002$ but not HF ($n = 10$) $r = 0.305$, $p = 0.391$; LD ($n = 1$).

When all patients were classified by volume status this altered the relationship between [Hb] & tHb-mass whereby the extent of volume expansion (BV and PV) reduced the association between [Hb] and tHb-mass. Specifically, hypovolemic patients ($n = 5$), $r = 0.89$, $p = 0.041$; normovolemic patients ($n = 31$), $r = 0.89$, $p < 0.0001$; mild to moderate BV hypervolemia ($n = 37$), $r = 0.77$, $p < 0.0001$; severe BV hypervolemia ($n = 36$), $r = 0.46$, $p = 0.004$). A similar trend was found for the extent of PV expansion, hypovolemic patients ($n = 3$), $r = 0.99$, $p = 0.066$; normovolemic patients ($n = 24$), $r = 0.93$, $p < 0.0001$; mild to moderate PV hypervolemia ($n = 36$), $r = 0.88$, $p < 0.0001$; severe PV hypervolemia ($n = 46$), $r = 0.54$, $p < 0.0001$). Where possible (due to small numbers in each sub

category), sub-group analysis was performed across patient groups for completeness: normovolemic IBD patients (n= 10), $r = 0.90$, $p < 0.0001$; mild to moderate PV hypervolemia (n= 7), $r = 0.92$, $p < 0.003$; severe PV hypervolemia (n= 5), $r = 0.54$, $p = 0.339$). Normovolemic surgical patients (n= 7), $r = 0.95$, $p = 0.001$; mild to moderate PV hypervolemia (n= 14), $r = 0.85$, $p < 0.0001$; severe PV hypervolemia (n= 5), $r = 0.824$, $p < 0.0001$). Normovolemic HF patients (n= 4), $r = 0.95$, $p = 0.046$; mild to moderate PV hypervolemia (n= 6), $r = 0.86$, $p < 0.029$; severe PV hypervolemia (n= 11), $r = 0.59$, $p = 0.05$). Mild to moderate PV hypervolemia in HV (n= 8), $r = 0.91$, $p < 0.002$; severe PV hypervolemia (n= 11), $r = 0.89$, $p < 0.0001$).

5.4.2 Linear regression models

In the whole population tHb-mass explained 25.0 % of the variance in [Hb] (adjusted $R^2 = 0.250$, $p < 0.0001$). PV independently accounted for 64.3 % of the variance in [Hb] over and above tHb-mass (R^2 change= 0.624, $p < 0.0001$), in the whole population. In sub-groups, tHb-mass explained different amounts of the variance in [Hb] (HV adjusted $R^2 = 0.746$, $p < 0.0001$; IBD adjusted $R^2 = 0.446$, $p < 0.0001$; surgical adjusted $R^2 = 0.605$, $p < 0.0001$). Of particular note, tHb-mass did *not* significantly explain variance in [Hb] in the two patient groups most likely to suffer expanded plasma volume and shifts in fluid- CLD (adjusted $R^2 = 0.109$, $p = 0.114$) or CHF patients (adjusted $R^2 = 0.052$, $p = 0.157$). In keeping, PV independently accounted for a greater proportion of the variance in [Hb] over and above tHb-mass in these group (R^2 change= 0.724 in CHF and 0.805 in CLD) than in HV (R^2 change= 0.192), surgical patients (R^2 change= 0.351) or IBD patients (R^2 change= 0.422) ($p < 0.0001$ in all cases). Together, tHb-mass and PV explained 94.5 % of the variance in [Hb] in HV and IBD patients (adjusted $R^2 = 0.945$, $p < 0.0001$), 96.9% in surgical patients (adjusted $R^2 = 0.969$, $p < 0.0001$), 96.9% in LD patients (adjusted $R^2 = 0.969$, $p < 0.0001$), and 80.3 % in patients with CHF (adjusted $R^2 = 0.803$, $p < 0.0001$).

5.4.3 Haemoglobin concentration, plasma volume and total haemoglobin mass in individual participants

Figure 5-4, Figure 5-5, Figure 5-6, Figure 5-7, and Figure 5-8 show individual participants ranked in order by weight adjusted total haemoglobin mass from smallest to largest with corresponding PV ($\text{ml}\cdot\text{kg}^{-1}$) and [Hb] ($\text{g}\cdot\text{l}^{-1}$) in HV, IBD, surgical, CHF and LD patients, respectively. These figures show that on an individual level, patients may have the same or very similar tHb-mass values but different [Hb] and related PV values. For example, in patients with IBD (Figure 5-5), patient number 17 and 18 have very similar tHb-mass values ($9.2\text{ g}\cdot\text{kg}^{-1}$ and $9.3\text{ g}\cdot\text{kg}^{-1}$, respectively) but [Hb] of $161\text{ g}\cdot\text{l}^{-1}$ and $107\text{ g}\cdot\text{l}^{-1}$, respectively, with an elevated PV in patient number 18 compared to 17 ($65.7\text{ ml}\cdot\text{kg}^{-1}$ versus $37.2\text{ ml}\cdot\text{kg}^{-1}$). Clinically these patients may be treated differently despite very minimal differences in O_2 carrying capacity (tHb-mass). Similarly, in LD patients (Figure 5-8), tHb-mass in patient number 2 and 3 are the same ($5.2\text{ g}\cdot\text{kg}^{-1}$) but [Hb] is substantially lower in patient number 3 (severely anaemic) compared to patient number 2 (moderately anaemic), ($69\text{ g}\cdot\text{l}^{-1}$ versus $110\text{ g}\cdot\text{l}^{-1}$; PV 67.8 versus $36.5\text{ ml}\cdot\text{kg}^{-1}$, respectively).

5.4.4 Blood, plasma and red cell volumes

Derived BV, PV and RCVs were greater than expected normal values in all patients (BV, 5717 ± 1426 vs. $4789 \pm 873\text{ ml}$, $p < 0.0001$; PV, 3650 ± 1022 vs. 2887 ± 464 , $p < 0.0001$; RCV, 2067 ± 582 vs. 1901 ± 410 , $p < 0.0001$) and across all sub-groups for BV and PV but not for RCV in LD patients (see Table 5-8). Differences existed across sub-groups in the extent of deviation from expected normal PV. Specifically, LD patients had the greatest deviation ($+61 \pm 38\%$) compared to IBD ($+13 \pm 16\%$, $p = 0.002$), surgical ($+13 \pm 16\%$, $p = 0.002$), and HV ($+26 \pm 15\%$, $p = 0.022$) but not HF ($+30 \pm 25\%$), although this was approaching significance ($p = 0.062$), see Table 5-8. There were no significant differences across sub-groups in the extent of deviation from expected normal RCV (ml).

When all patients were categorised by the extent of PV expansion, defined as normal when derived PV was within $\pm 8\%$ of the expected normal volume on an individual level, mild to moderate volume expansion was considered $>8\%$ to $<25\%$ deviation from expected norms, and severe as $>25\%$ of the expected normal volume, differences in [Hb] and Hct were found, as shown in Figure 5-10 (A-B). Specifically, in patients with severe PV expansion, [Hb] was significantly lower ($119.3 \pm 17.9 \text{ g}\cdot\text{l}^{-1}$) than patients with mild to moderate PV expansion ($131.9 \pm 14.3 \text{ g}\cdot\text{l}^{-1}$, $p= 0.005$), normal PV ($140.3 \pm 17.6 \text{ g}\cdot\text{l}^{-1}$, $p< 0.0001$) and PV contraction ($151.9 \pm 9.7 \text{ g}\cdot\text{l}^{-1}$, $p= 0.001$), see Figure 5-10 (A). Similarly, Hct (%) was significantly reduced in patients with severe PV expansion ($37.2 \pm 6.4 \%$) compared to patients with mild to moderate PV expansion ($40.8 \pm 3.9 \%$, $p= 0.014$) and normal PV ($42.7 \pm 4.1 \%$, $p< 0.0001$). Patients with PV contraction ($n=3$) had numerically elevated Hct ($46.0 \pm 3.5\%$) compared to severe PV expansion group, although this was not statistically significant ($p= 0.086$), Figure 5-10 (B).

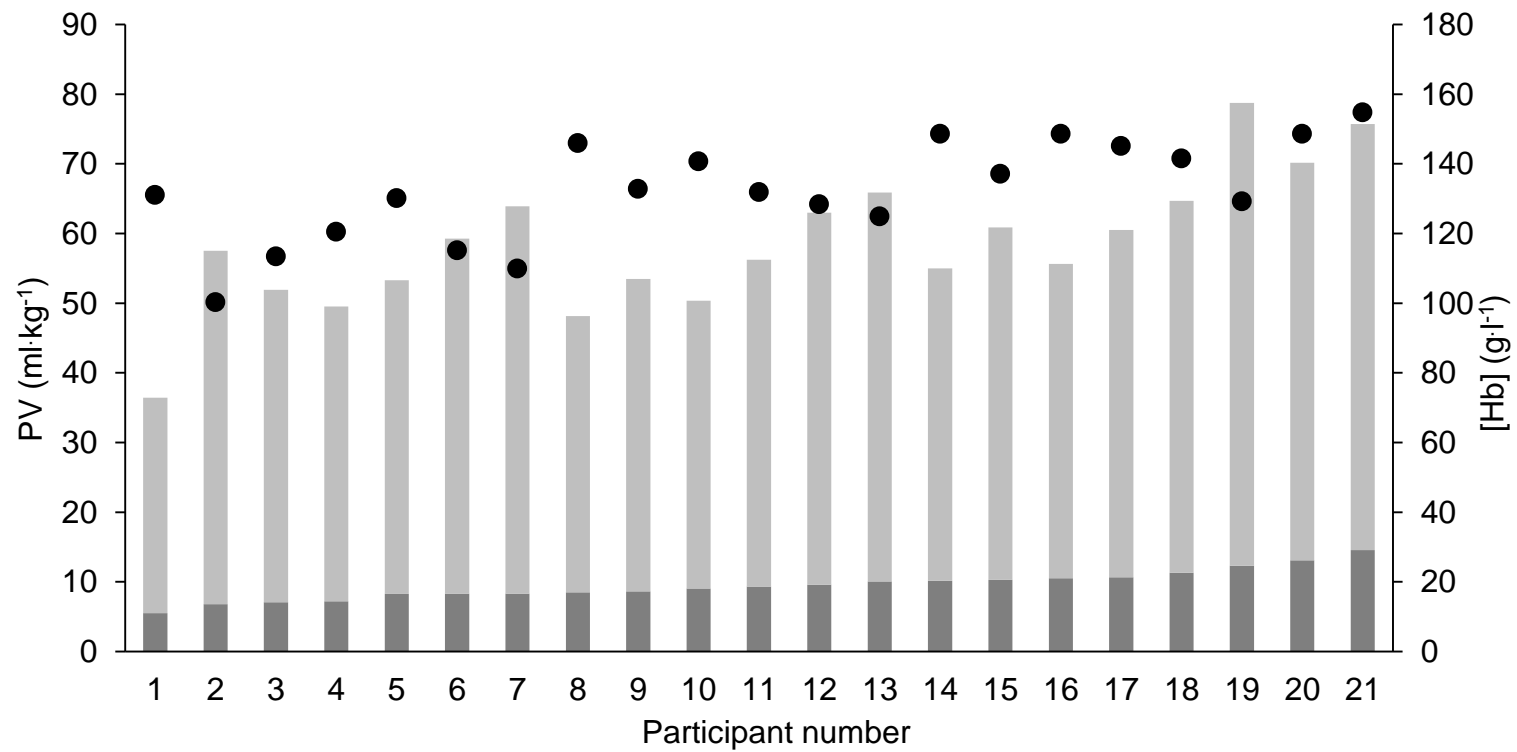


Figure 5-4. Individual participants ranked by tHb-mass ($\text{g}\cdot\text{kg}^{-1}$) (dark grey bars) with corresponding PV ($\text{ml}\cdot\text{kg}^{-1}$) (light grey bars) and [Hb] ($\text{g}\cdot\text{l}^{-1}$) (black circles) in healthy controls ($n=21$). tHb-mass, total haemoglobin mass; PV, plasma volume; [Hb], haemoglobin concentration.

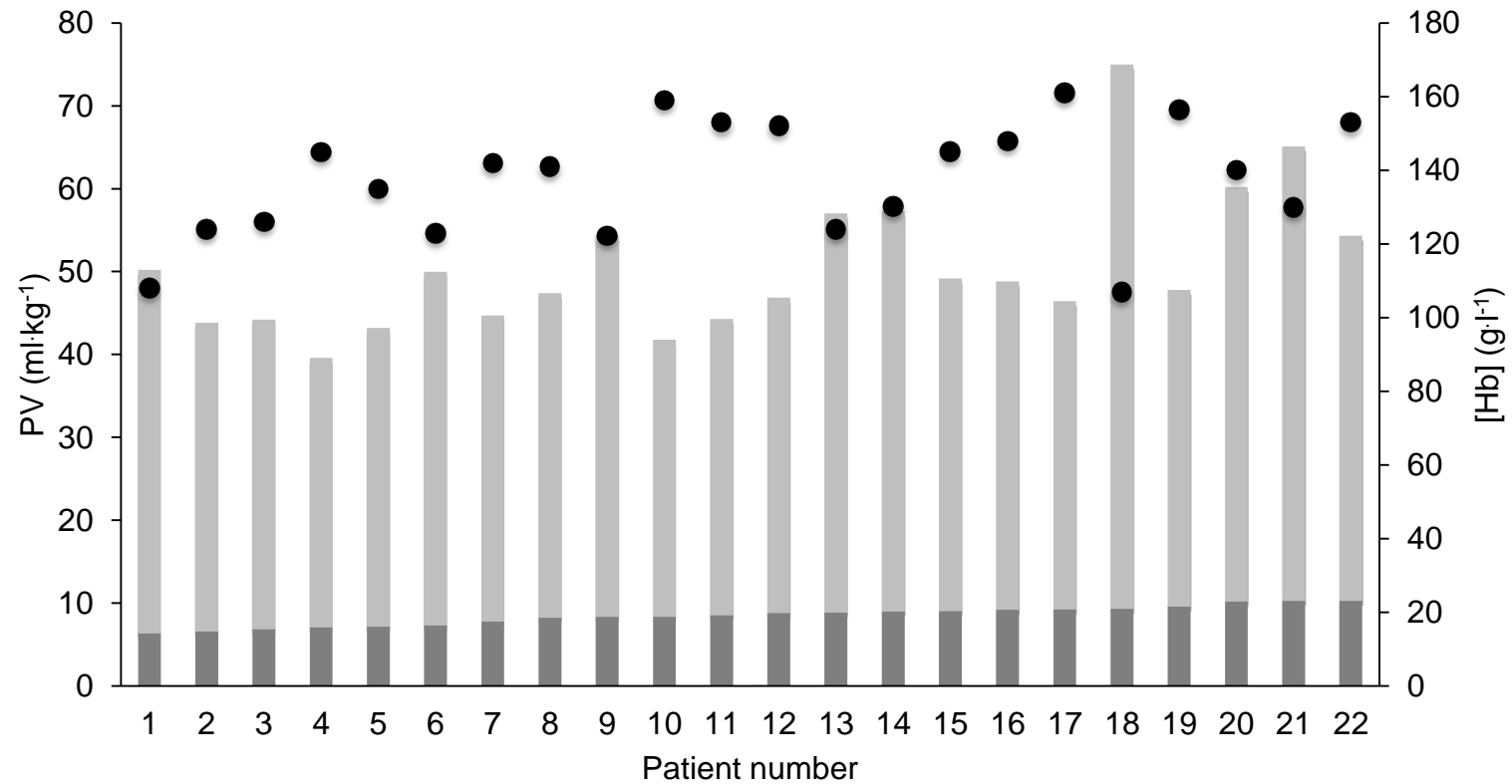


Figure 5-5. Individual IBD patients (n= 22) ranked by tHb-mass ($\text{g}\cdot\text{kg}^{-1}$) (dark grey bars) with corresponding PV ($\text{ml}\cdot\text{kg}^{-1}$) (light grey bars) and [Hb] ($\text{g}\cdot\text{l}^{-1}$) (black circles). tHb-mass, total haemoglobin mass; PV, plasma volume; [Hb], haemoglobin concentration.

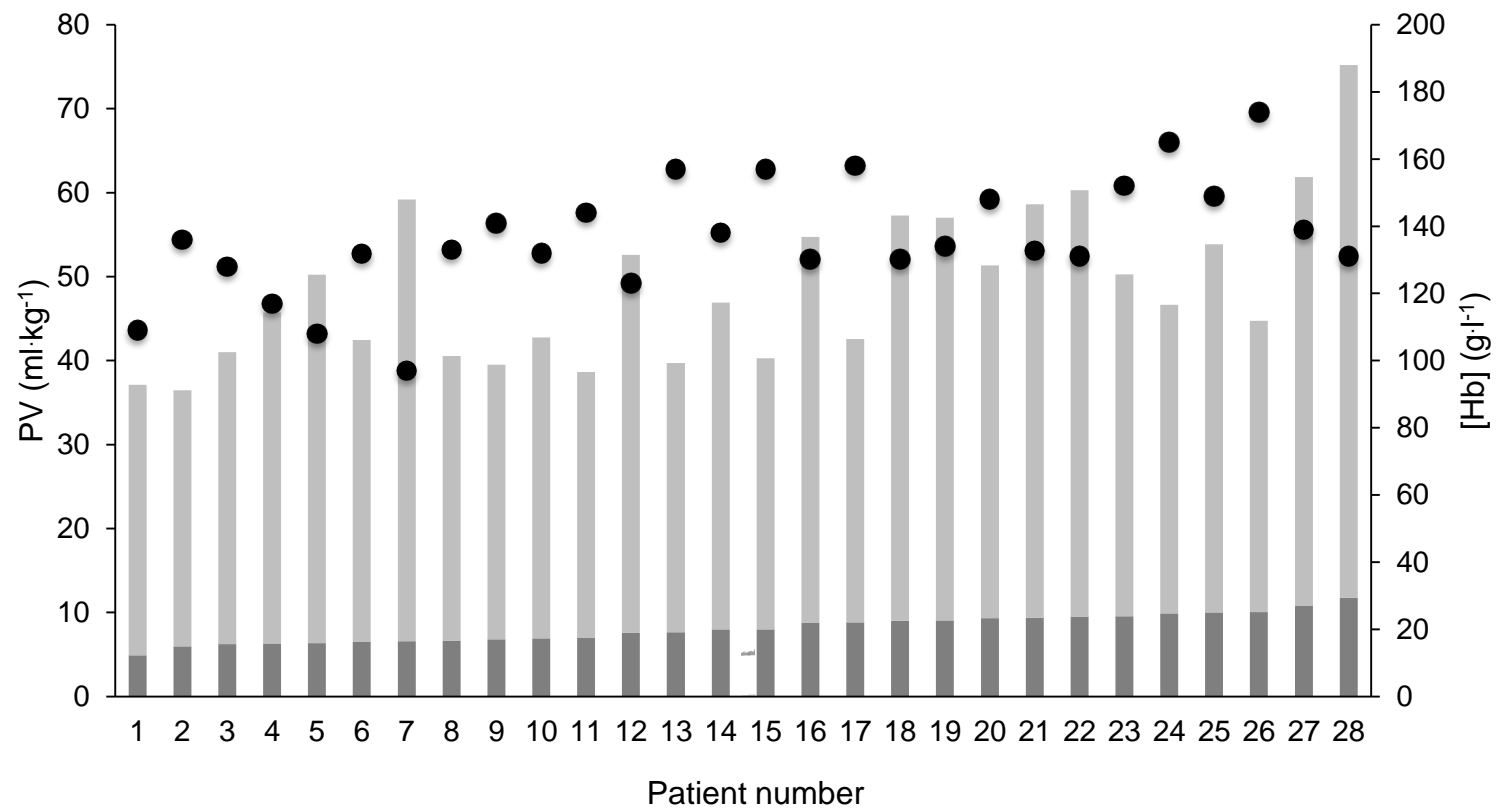


Figure 5-6. Individual surgical patients (n= 28) ranked by tHb-mass ($\text{g}\cdot\text{kg}^{-1}$) (dark grey bars) with corresponding PV ($\text{ml}\cdot\text{kg}^{-1}$) (light grey bars) and [Hb] ($\text{g}\cdot\text{l}^{-1}$) (black circles). tHb-mass, total haemoglobin mass; PV, plasma volume; [Hb], haemoglobin concentration.

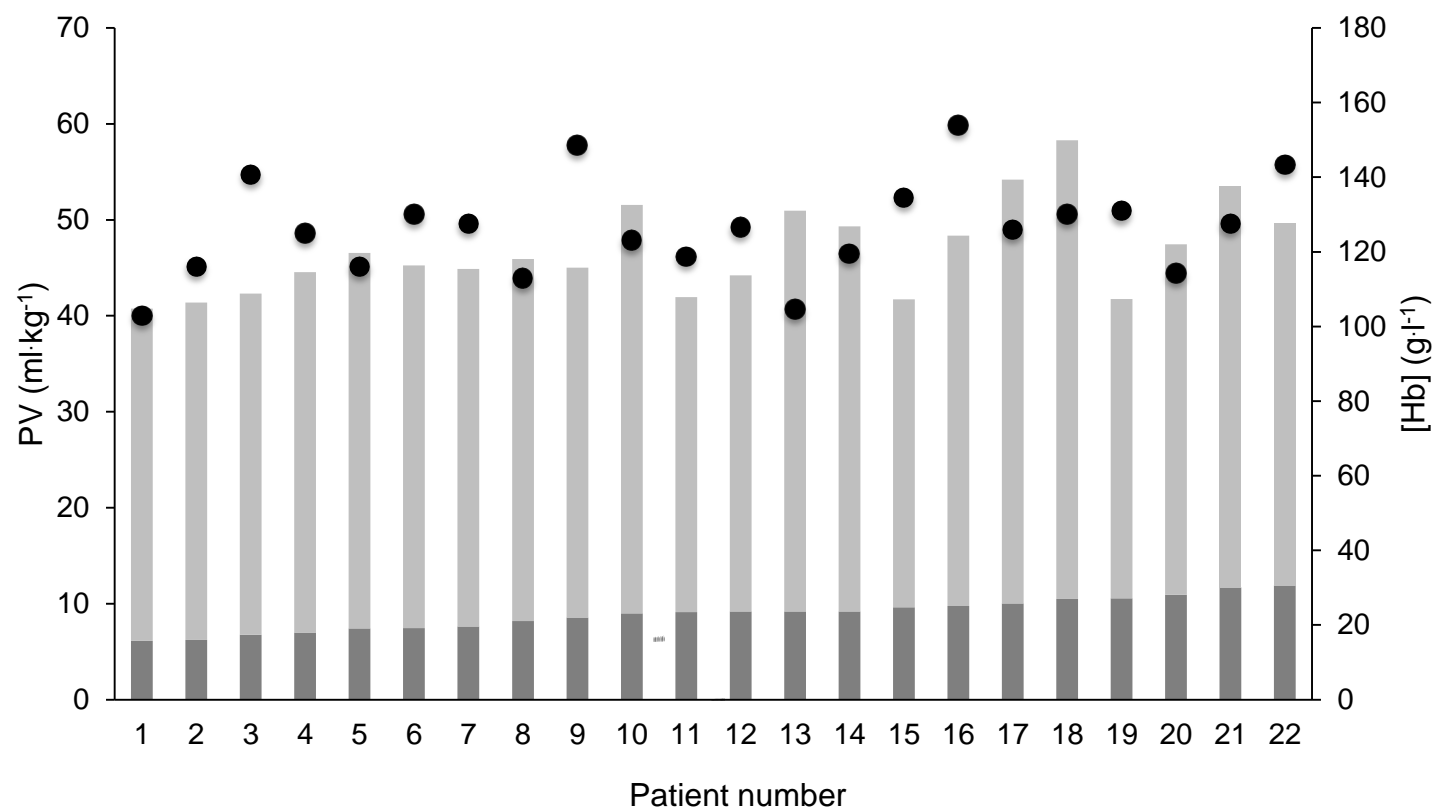


Figure 5-7. Individual chronic heart failure patients (n= 22) ranked by tHb-mass ($\text{g}\cdot\text{kg}^{-1}$) (dark grey bars) with corresponding PV ($\text{ml}\cdot\text{kg}^{-1}$) (light grey bars) and [Hb] ($\text{g}\cdot\text{l}^{-1}$) (black circles). tHb-mass, total haemoglobin mass; PV, plasma volume; [Hb], haemoglobin concentration.

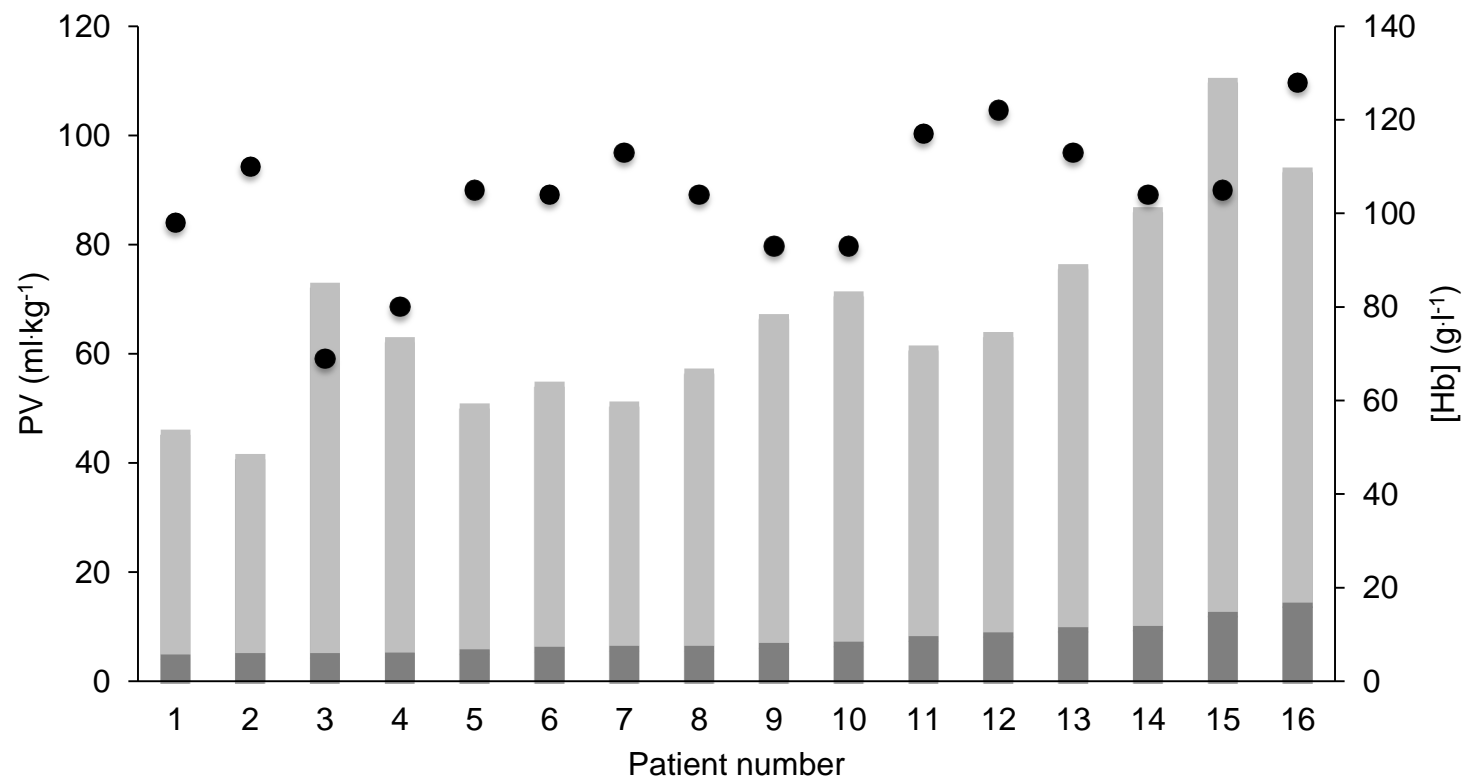
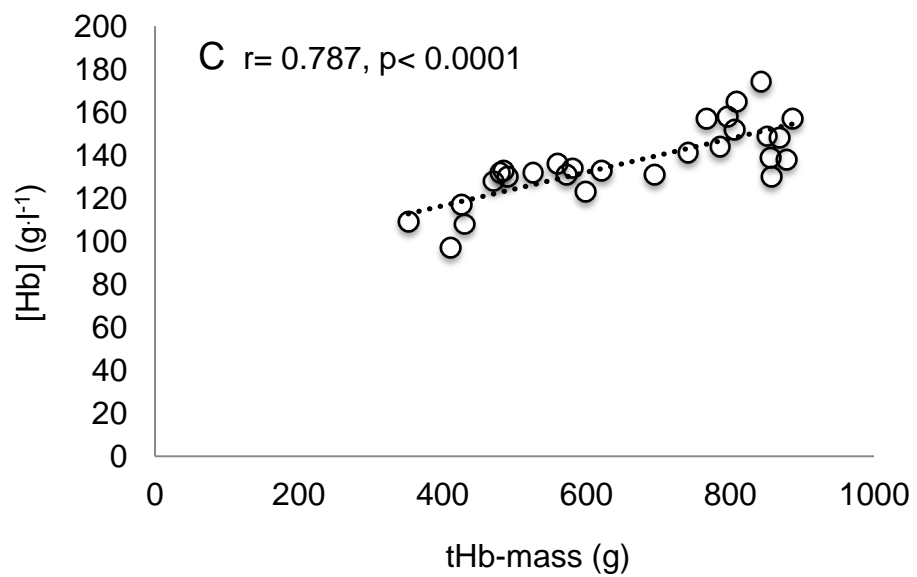
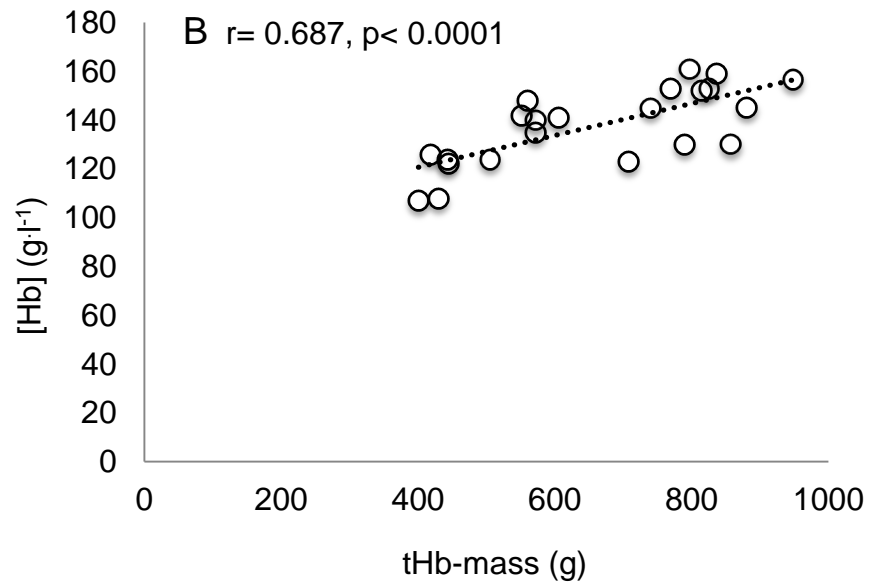
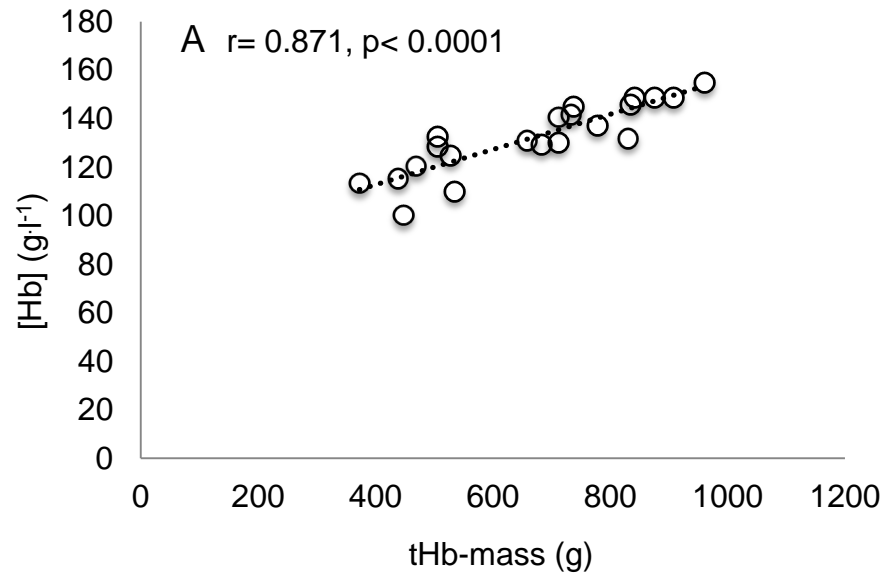


Figure 5-8. Individual chronic liver disease patients (n= 16) ranked by tHb-mass ($\text{g}\cdot\text{kg}^{-1}$) (dark grey bars) with corresponding PV ($\text{ml}\cdot\text{kg}^{-1}$) (light grey bars) and [Hb] ($\text{g}\cdot\text{l}^{-1}$) (black circles). tHb-mass, total haemoglobin mass; PV, plasma volume; [Hb], haemoglobin concentration.



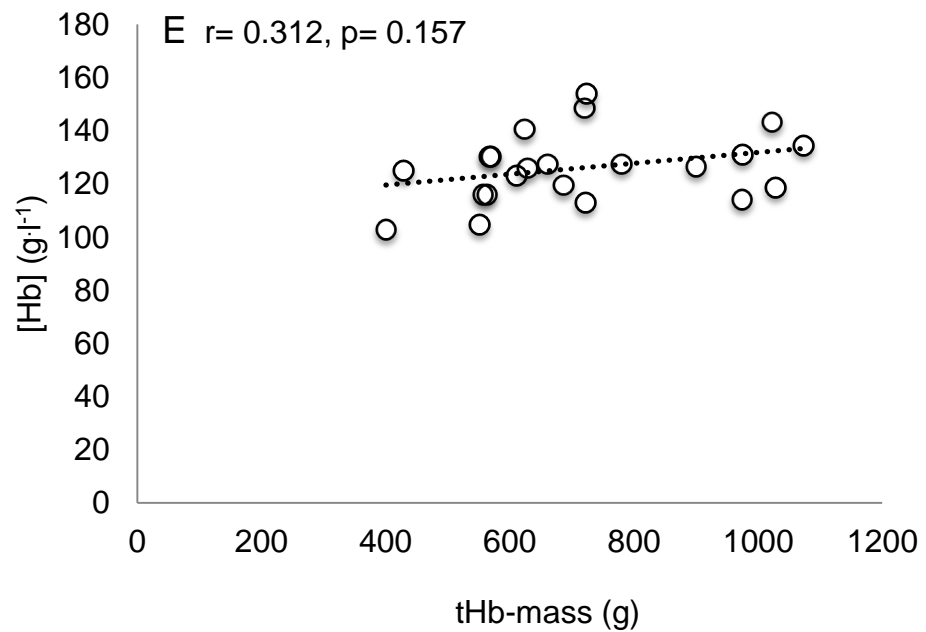
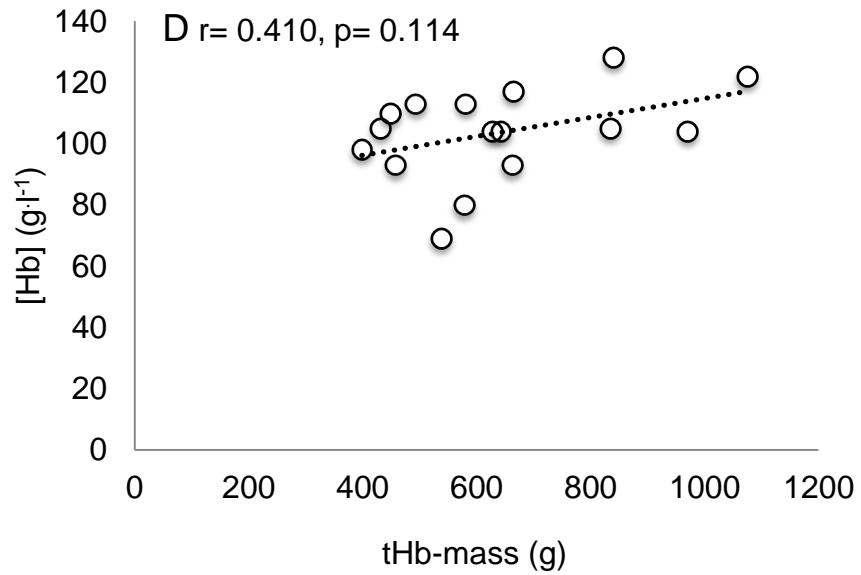


Figure 5-9. Unadjusted relationship between [Hb] (g·l⁻¹) and tHb-mass (g) in healthy controls (A, n= 21), patients with IBD (B, n= 22), surgical patients (C, n= 28), liver disease (D, n= 16) and CHF (E, n= 22). tHb-mass, total haemoglobin mass; [Hb], haemoglobin concentration; IBD, inflammatory bowel disease; CHF, chronic heart failure.

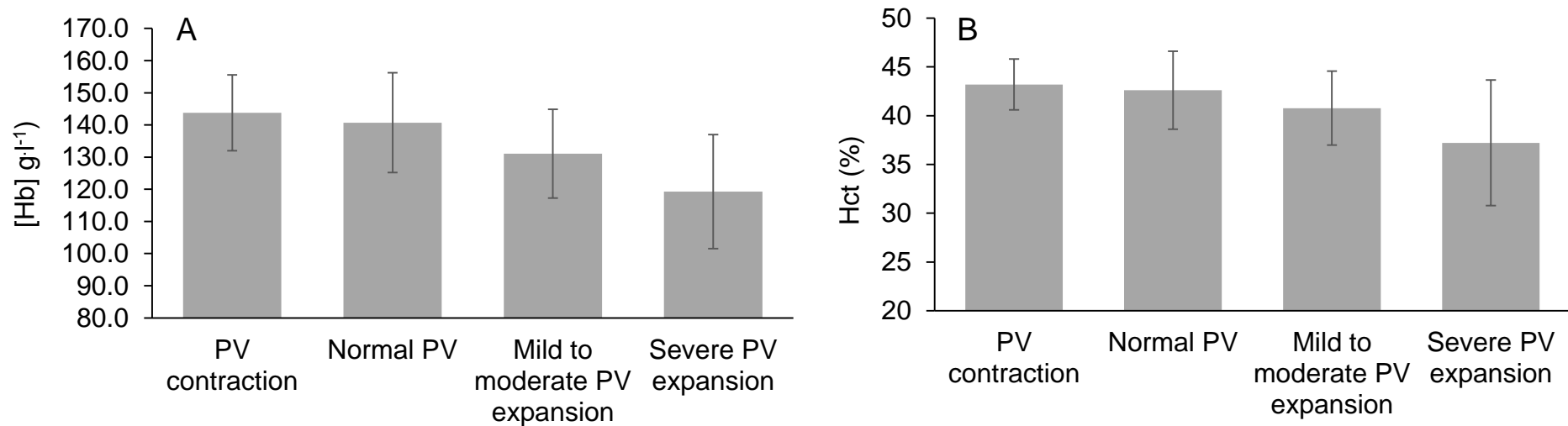


Figure 5-10. Haemoglobin concentration (A) and haematocrit (B) in all patients categorised by plasma volume (PV) status. PV contraction (n= 3) was classified as > minus 8% from expected norm. Normal PV (n= 24) was classified as derived PV within $\pm 8\%$ of the expected normal volume on an individual level. Mild to moderate volume expansion (n= 36) was considered >8% to <25% deviation from expected norms, and severe PV expansion (n= 46) as >25% of the expected normal volume.

Table 5-1. Surgical specialty of planned surgical procedure.

Surgical specialty	Frequency N (%)
Upper gastrointestinal	8 (28.5%)
Lower gastrointestinal	8 (28.5%)
Orthopaedic	7 (25%)
Other	3 (10.7%)
Urology	2 (7.1%)

Table 5-2. Characteristics and aetiology of inflammatory bowel disease patients.

Variable	IBD (n= 22)
<i>Gender</i>	
Male (%)	50%
Age (yr)	50 (33-62)
Height (cm)	170.2 ± 7.3
Weight (kg)	77.7 ± 18.1
<i>Aetiology of IBD</i>	
Crohn's disease	4 (18%)
Small bowel CD	2 (9%)
Terminal ileal CD	1 (4.5%)
Ulcerative colitis	9 (41%)
Ulcerative proctitis	3 (14%)
Pan UC	2 (9%)
Collagenous colitis	1 (4.5%)

IBD, Inflammatory bowel disease; CD, Crohn's disease; UC, Ulcerative colitis

Table 5-3. Characteristics, medications and aetiology of liver disease patients.

Variable	LD (n= 16)
<i>Gender</i>	
Male (%)	75%
Age (yr)	51 (48-55)
Height (cm)	174.8 ± 8.9
Weight (kg)	85.3 ± 18.5
<i>Primary aetiology of liver disease</i>	
Hepatitis	3 (19%)
ALD	8 (50%)
Cryptogenic cirrhosis	2 (12%)
Sclerosing cholangitis	2 (12%)
Other	1 (6%)
Hypertension	1 (6%)
<i>Medication</i>	
Beta blocker	3 (19%)
ACE inhibitor	0 (0%)
Statin	0 (0%)
Diuretic	5 (31%)
<i>Diuretic times per day</i>	
None	11 (69 %)
Once	5 (31 %)
Bi daily	0
ALD, decompensated alcoholic liver disease; ACE inhibitor, angiotensin-converting-enzyme inhibitor.	

Table 5-4. Characteristics, medications and aetiology of chronic heart failure patients.

Variable	CHF (n= 22)
<i>Gender</i>	
Male (%)	82%
Age (yr)	68 (61-75)
Height (cm)	173.5 ± 8.1
Weight (kg)	80.4 ± 15.9
<i>Aetiology of heart failure</i>	
Ischaemic heart disease	7 (32%)
Cardiomyopathy	10 (45%)
Ischaemic	5 (22%)
Non-ischaemic	5 (22%)
Other	5 (22%)
Hypertension	7 (32%)
<i>NHYA class</i>	
I	2 (9%)
II	14 (64%)
III	4 (18%)
IV	2 (9%)
LVEF (%)	33.2 ± 11.4
<i>Medication</i>	
Beta blocker	19 (86%)
ACE inhibitor	20 (91%)
Statin	16 (73%)
Diuretic	17 (77%)
Diuretic dose (mg)	25 ± 29
<i>Diuretic times per day</i>	
None	5 (22%)
Once	14 (64%)
Bi daily	3 (14 %)

NYHA, New York Heart Association Class; LVEF (%), left ventricular ejection fraction; ACE inhibitor, angiotensin-converting-enzyme inhibitor.

Table 5-5. Haematological variables in males and females.

Variable	Whole group (n= 109)	Males (n= 66)	Females (n= 43)	P-value
[Hb] (g·l ⁻¹)	128.9 ± 18.8	132.8 ± 20.9	123.0 ± 13.1	0.003
Anaemia (%)	36%	35%	37%	
Hct (%)	39.8 ± 5.6	40.5 ± 6.6	38.7 ± 3.4	0.057
tHb-mass (g)	669 ± 181	758 ± 152	533 ± 132	< 0.0001
tHb-mass (g·kg ⁻¹)	8.5 ± 1.9	8.9 ± 1.8	7.9 ± 2.0	0.006
tHb-mass (g·m ²)	348 ± 74	375 ± 67	305 ± 65	< 0.0001
BV (ml)	5717 ± 1426	6346 ± 1325	4754 ± 970	< 0.0001
BV (ml·kg ⁻¹)	73.4 ± 15.7	74.9 ± 14.5	71.1 ± 17.2	0.222
BV(ml·m ²)	2980 ± 570	3140 ± 552	2733 ± 511	< 0.0001
PV (ml)	3650 ± 1022	4021 ± 1053	3080 ± 649	< 0.0001
PV (ml·kg ⁻¹)	46.9 ± 11.4	47.4 ± 11.5	46.1 ± 11.5	0.556
PV (ml·m ²)	1902 ± 427	1988 ± 455	1771 ± 347	0.009
PV (%)	64%	63%	65%	0.057
RCV (ml)	2067 ± 582	2324 ± 552	1673 ± 368	< 0.0001
RCV (ml·kg ⁻¹)	26.5 ± 6.3	27.4 ± 6.2	25.0 ± 6.3	0.049
RCV (ml·m ²)	1077 ± 244	1151 ± 246	962 ± 192	< 0.0001

[Hb], Haemoglobin concentration; Hct, haematocrit; tHb-mass, total haemoglobin mass; BV, blood volume; PV, plasma volume; RCV, red cell volume. Data are presented as mean ± SD, or frequency (%).

Table 5-6. Haematological variables in anaemic and non-anaemic participants.

Variable	Whole group (n= 109)	Anaemic (n= 39)	Non-anaemic (n= 70)	P-value
[Hb] (g·l ⁻¹)	128.9 ± 18.8	109.6 ± 12.5	138.9 ± 10.6	< 0.0001
Anaemia (%)	36%			
Hct (%)	39.8 ± 5.6	34.9 ± 5.9	42.4 ± 3.0	< 0.0001
tHb-mass (g)	669 ± 181	594 ± 192	711 ± 162	0.001
tHb-mass (g·kg ⁻¹)	8.5 ± 1.9	7.7 ± 1.9	9.0 ± 1.8	0.001
tHb-mass (g·m ²)	348 ± 74	311 ± 76	368 ± 65	< 0.0001
BV (ml)	5717 ± 1426	5977 ± 1837	5606 ± 1146	0.217
BV (ml·kg ⁻¹)	73.4 ± 15.7	77.3 ± 16.7	71.6 ± 14.8	0.051
BV(ml·m ²)	2980 ± 570	3123 ± 692	2900 ± 476	0.078
PV (ml)	3650 ± 1022	4083 ± 1351	3434 ± 686	0.005
PV (ml·kg ⁻¹)	46.9 ± 11.4	52.6 ± 12.2	44.0 ± 9.6	< 0.0001
PV (ml·m ²)	1902 ± 427	2130 ± 517	1776 ± 305	< 0.0001
PV (%)	64%	68%	61%	< 0.0001
RCV (ml)	2067 ± 582	1894 ± 692	2171 ± 501	0.018
RCV (ml·kg ⁻¹)	26.5 ± 6.3	24.7 ± 7.1	27.5 ± 5.6	0.023
RCV (ml·m ²)	1077 ± 244	993 ± 293	1123 ± 199	0.007
MCV (fl)	89.8 ± 6.5	88.0 ± 6.2	91.0 ± 6.6	0.038
MCH (pg)	29.8 ± 2.5	29.3 ± 2.9	30.1 ± 2.2	0.218
MCHC (g·l ⁻¹)	331.6 ± 14.7	333.1 ± 18.0	330.5 ± 12.2	0.477
RDW (%)	13.9 ± 1.4	14.5 ± 1.7	13.7 ± 1.1	0.024
Creatinine (μmol·l ⁻¹)	96.7 ± 87.8	114.9 ± 117.4	84.3 ± 58.2	0.166
Albumin (g·l ⁻¹)	41.1 ± 7.4	37.4 ± 8.7	43.7 ± 4.9	0.001

[Hb], Haemoglobin concentration; Hct, haematocrit; tHb-mass, total haemoglobin mass; BV, blood volume; PV, plasma volume; RCV, red cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; RDW, red cell distribution width. Anaemia defined according to World Health Organisation criteria ([Hb] <130 g·l⁻¹ in men and <120 g·l⁻¹ in women. Data are presented as mean ± SD, or frequency (%).

Table 5-7. Haematological indices for the whole population and in patient sub-groups.

Variable	Whole group (n= 109)	HV (n= 21)	IBD (n= 22)	Surgical (n= 28)	LD (n= 16)	CHF (n= 22)
[Hb] (g·l ⁻¹)	128.9 ± 18.8	132.2 ± 14.4	137.5 ± 15.7	136.6 ± 17.3	103.6 ± 14.9	126.1 ± 12.9
Anaemia (%)	36%	24%	9%	18%	94%	54%
Hct (%)	39.8 ± 5.6	41 ± 3.2	42.3 ± 3.4	41.6 ± 4.1	30.0 ± 4.8	41.2 ± 4
tHb-mass (g)	669 ± 181	670 ± 175	657 ± 175	658 ± 174	640 ± 198	716 ± 197
tHb-mass (g·kg ⁻¹)	8.5 ± 1.9	9.5 ± 2.1	8.4 ± 1.2	8.1 ± 1.6	7.7 ± 2.8	8.9 ± 1.6
tHb-mass (g·m ²)	348 ± 74	366 ± 77	344 ± 59	337 ± 66	321 ± 98	367 ± 74
BV (ml)	5717 ± 1426	5496 ± 990	5208 ± 1078	5239 ± 1007	6811 ± 1860	6252 ± 1659
BV (ml·kg ⁻¹)	73.4 ± 15.7	78.5 ± 13.4	68.2 ± 10.3	65.4 ± 12	81.6 ± 23.1	77.9 ± 14.3
BV (ml·m ²)	2980 ± 570	3025 ± 397	2749 ± 345	2703 ± 371	3401 ± 829	3213 ± 618
PV (ml)	3650 ± 1022	3429 ± 538	3189 ± 635	3249 ± 571	4965 ± 1447	3883 ± 953
PV (ml·kg ⁻¹)	46.9 ± 11.4	49.1 ± 7.9	42 ± 7.7	40.7 ± 8.2	59.1 ± 16.0	48.6 ± 9.2
PV (ml·m ²)	1902 ± 427	1890 ± 207	1688 ± 234	1678 ± 236	2471 ± 615	2001 ± 362
PV (%)	64%	63%	61%	62%	73%	62%
RCV (ml)	2067 ± 582	2067 ± 487	2018 ± 487	1997 ± 495	1845 ± 565	2368 ± 770
RCV (ml·kg ⁻¹)	26.5 ± 6.3	29.4 ± 6.2	26.1 ± 3.5	24.6 ± 4.8	22.5 ± 8.4	29.3 ± 6.5
RCV (ml·m ²)	1077 ± 244	1135 ± 214	1060 ± 155	1024 ± 185	930 ± 290	1212 ± 301

[Hb], Haemoglobin concentration; Hct, haematocrit; tHb-mass, total haemoglobin mass; BV, blood volume; PV, plasma volume; RCV, red cell volume; HV, healthy volunteers; IBD, inflammatory bowel disease; LD, liver disease; CHF, chronic heart failure. Anaemia defined according to World Health Organisation criteria ([Hb] <130 g·l⁻¹ in men and <120 g·l⁻¹ in women. Values are expressed as mean ± SD, or frequency (%).

Table 5-8. Derived blood, plasma and red cell volumes and expected norms in all participants and patient sub-groups.

Variable	Whole group (n= 109)	HV (n= 21)	IBD (n= 22)	Surgical (n= 28)	LD (n= 16)	CHF (n= 22)
Derived BV (ml)	5717 ± 1426 ^a	5496 ± 990 ^a	5208 ± 1078 ^a	5239 ± 1007 ^a	6811 ± 1860 ^c	6252 ± 1659 ^d
Expected normal BV (ml)	4789 ± 873	4487 ± 797	4666 ± 939	4749 ± 884	5164 ± 920	4979 ± 752
Deviation from expected normal BV (%)	+19 ± 21%	+23 ± 15%	+12 ± 12%	+11 ± 14%	+33 ± 33%	+25 ± 25%
Derived PV (ml)	3650 ± 1022 ^a	3429 ± 538 ^a	3189 ± 635 ^a	3249 ± 571 ^a	4965 ± 1447 ^b	3883 ± 953 ^e
Expected normal PV (ml)	2887 ± 464	2730 ± 432	2818 ± 502	2874 ± 471	3079 ± 483	2985 ± 399
Deviation from expected normal PV (%)	+26 ± 27%	+26 ± 15%	+13 ± 16%	+13 ± 16%	+61 ± 38%	+30 ± 25%
Derived RCV (ml)	2067 ± 582 ^a	2067 ± 487 ^a	2018 ± 487 ^d	1997 ± 495 ^b	1845 ± 565	2368 ± 770 ^f
Expected normal RCV (ml)	1901 ± 410	1757 ± 367	1847 ± 439	1875 ± 416	2085 ± 437	1994 ± 354
Deviation from expected normal RCV (%)	+9 ± 23%	+18 ± 17%	+9 ± 11%	+6 ± 15%	-8 ± 34%	+18 ± 27%

Data expressed as mean ± SD. Blood volume (BV) derived from quantified total haemoglobin mass using the formula: tHb-mass (g)/[Hb] (g·dl⁻¹) • 100. Plasma volume (PV) calculated using the formula: PV (ml) = BV – RCV. Red cell volume (RCV) calculated using the formula: RCV (ml) = tHbmass (g) /MCHC (g·dl⁻¹) • 100. ^ap< 0.0001, ^bp= 0.022, ^cp= 0.041, ^dp= 0.001, ^ep= 0.002, ^fp= 0.007 derived vs. expected volumes. MCHC, mean corpuscular haemoglobin concentration.

Table 5-9. Anaemia based on haemoglobin concentration, red cell and plasma volume status in all participants (n= 109).

Anaemia status		Normal PV	Mild to moderate PV expansion	Severe PV expansion	PV contraction	Total
Non-anaemic	Normal RCV	12	8	1	1	22
	RCV deficit	4	1	0	2	7
	RCV excess	5	17	19	0	41
	Total	21	26	20	3	70
Anaemic	Normal RCV	1	5	5	0	11
	RCV deficit	2	4	8	0	15
	RCV excess	0	1	13	0	14
	Total	3	10	26	0	39

Normal BV, PV and RCV were classified as derived volumes from measured tHb-mass within $\pm 8\%$ of the expected normal volumes on an individual level. Mild to moderate volume expansion was considered $>8\%$ to $<25\%$ deviation from expected norms, and severe as $>25\%$ of the expected normal volume. BV, blood volume, PV, plasma volume, RCV, red cell volume. Anaemia defined according to World Health Organisation criteria ($[\text{Hb}] < 130 \text{ g}\cdot\text{l}^{-1}$ in men and $< 120 \text{ g}\cdot\text{l}^{-1}$ in women. Values are expressed as counts.

5.5 Discussion

To my knowledge this is one of the largest studies to have measured total haemoglobin mass in the clinical setting through the novel application of the optimised carbon monoxide rebreathing method. The aim of this study was to explore the relationship between total haemoglobin mass and haemoglobin concentration in different patient populations to ascertain the degree to which tHb-mass correlated with [Hb] and the contribution of tHb-mass and PV to measured [Hb]. It was hypothesised that tHb-mass and [Hb] would be poorly correlated and therefore [Hb] a poor index of O₂ carrying capacity.

I found a prevalence of anaemia of 36% (using WHO criteria) in all participants, although this varied considerably across sub-groups, ranging from 9% in IBD patients to 94% in patients with chronic LD. The key findings from this study were as follows; there was a moderate relationship between tHb-mass and [Hb] in all participants ($r = 0.500$, $p < 0.0001$, $n = 109$) but this varied considerably across sub-groups and appeared to degrade with disease severity in so much that tHb-mass was strongly related to [Hb] in HV ($r = 0.871$, $p < 0.0001$), surgical ($r = 0.787$, $p < 0.0001$) and IBD ($r = 0.687$, $p < 0.0001$) patients. However, in CHF and LD patients tHb-mass did not correlate with [Hb], and tHb-mass explained no significant variance in [Hb].

In the whole population, PV independently accounted for 64% of the variance in [Hb] over and above that attributable to tHb-mass, although again this varied considerably across sub-groups. Specifically, the degree to which PV influenced [Hb] independently of tHb-mass was greatest in those patient groups most likely to suffer expanded plasma volume and shifts in fluid- CHF (72%) and CLD (80%) patients compared to 19% in HV. No significant differences in tHb-mass (g, g·kg⁻¹ or g·m²) between sub-groups were found, although this was not the case for [Hb] and Hct. Specifically, [Hb] and Hct (%) were significantly lower in chronic LD patients compared to all other patient groups, related to differences in PV with wide inter-individual variability in PV observed. Taken together, these results highlight that relying on [Hb] alone as a guide to O₂ carrying capacity without knowledge of PV and tHb-mass may be limited.

Furthermore, it challenges the convention that [Hb] provides a good surrogate of tHb-mass. For example, an abnormal [Hb] when reduced could primarily be related to a low tHb-mass (impaired O₂ carrying capacity) in the setting of a 'normal' and stable PV, but could also be reduced in the context of PV expansion without any deficit in tHb-mass. I have shown a progressive decrease in [Hb] and Hct depending on the extent of PV expansion with PV exerting greatest effect on [Hb] in CHF and LD patients. My results suggest that changes in plasma volume in patients with chronic heart failure or liver disease (but which might occur across a range of other disease states) may strongly influence [Hb], leading to diagnoses of severe anaemia in patients whose tHb-mass is quite normal.

The overall prevalence of anaemia found in this study (36%) is similar to previous studies in non-cardiac surgical patients (30.4%) [13], CHF (34%) [158] but less than patients with chronic LD (50-75%) [166, 167]. In sub-groups, 9% of IBD patients suffered anaemia, which is less than has been reported across European Countries (24%) [7]. Some 54% of CHF patients suffered anaemia in the current study, somewhat more than has been previously reported in the Study of Anemia in a Heart Failure Population (STAMINA-HFP) Registry (34%) [158], and 30% in patients with advanced HF (NYHA class III or IV) [12]. However, our results are in keeping with other studies that have reported the prevalence of anaemia in CHF of 55.6% [317] and 61% [97] respectively. Of CLD patients, 94% suffered anaemia- a figure somewhat higher than that previously reported in some studies (50-75%) [166, 167], but is in keeping with data in decompensated CLD (86%) [318] or hepatitis C infection (75%) [165]. Anaemia was also found in five female healthy volunteers (24%) in the current study, a surprising finding given that this group was free from known chronic disease or use of regular medications. A possible explanation is menstruation leading to anaemia, although this cannot be confirmed in the current data. However, the menstrual cycle is the leading cause of iron deficiency, being the primary cause in approximately half of those with anaemia [319]. Such a figure is in keeping with the global prevalence of anaemia in the general population of non-pregnant females (30%) [1], but higher than 16% reported in non-pregnant women aged 15-49 years from high income regions and 22% in menstruating women from central and eastern Europe [320]. This may also be related to

volunteering bias in the current study whereby those who thought they might be anaemic preferentially applied to participate.

The aetiology of anaemia was not studied in Chapter 5 but this would have been of interest to ascertain given the patient populations studied. For example, anaemia of chronic inflammation, being inflammatory driven is the second most common cause of anaemia after iron deficiency [175] both of which are commonly encountered features of anaemia in IBD [321, 322], CHF [323, 324] and in patients preoperatively [304, 325]. In addition, anaemia is a common feature of chronic kidney disease (CKD) [326], with CKD a common comorbidity in CHF [327], being a strong independent risk factor for anaemia [300].

There appears to be a dearth of previous studies that have quantified tHb-mass in the clinical setting using the oCOR method [48, 49, 50]. Instead, previous research that has addressed whether [Hb] or Hct provides a good estimate of O₂ carrying capacity have measured RCV either directly by radiolabelling RBCs or indirectly through the quantification of PV. In 40 haematologically normal control subjects, Huber and colleagues [328] found a strong correlation between Hct (%) in the 20-50% range and RCV ($r = 0.880$, $p < 0.001$) determined by ⁵¹Cr labelling of RBCs. However, this relationship was disturbed when Hct (%) was reduced ($< 20\%$) and elevated above 50% in patients with polycythaemia. The authors found that in polycythaemia, the correlation between Hct and RCV lessened owing to wider variability in PV and BV. This suggests that direct measurement of RCV is required to provide sufficient accuracy in the diagnosis of polycythaemia, a finding supported by others using radiolabelled techniques [329, 330] and when applying the oCOR method [48]. Ahlgrim and colleagues confirmed that BV in patients with polycythaemia vera is expanded not only through elevated RCV but also as a result of PV expansion when compared to healthy controls.

The focus of such studies differed from mine: namely, they sought to address the degree to which variation in PV altered the accuracy of the diagnosis of polycythaemia, whilst I assessed the influence of variation in tHb-mass and PV on [Hb] per se and on the diagnosis of anaemia. Nor have any studies in this

field been comprehensive across disease states, or assessed tHb-mass (rather than RCV).

Nonetheless, others have investigated whether peripheral Hct is an accurate surrogate of RCV in critically ill surgical patients [331] and the limitations of Hct to assess the need for RBC transfusion in hypovolemic anaemic patients [332]. Takanishi and colleagues studied patients admitted to a surgical intensive care unit. PV was directly measured using I¹³¹ labelled albumin with RCV calculated from PV and peripheral Hct. The key finding was that Hct did not reflect true RCV with values deviating by as much as 15 Hct percentage points. Results may have been confounded by increased measurement error in Hct (which was not quantified in this study), given this is used to calculate RCV. In addition, as only PV was directly measured, any error in this technique will affect subsequent estimation of RCV and BV. Indeed, when I¹³¹ labelled albumin is used to measure PV, PV may be overestimated due to distribution into the extravascular space [333]. Nonetheless, knowledge of Hct, RCV and PV may offer additional information to allow a refined approach to transfusion therapy in surgical patients, although this remains to be confirmed.

The other main setting in which haematological variables have been studied in the context of anaemia and volume status is CHF. In a cohort of 100 patients, Adlbrecht and colleagues [96] showed no differences in quantified RCV determined by the ⁵¹Cr labelled RBC technique in anaemic and non-anaemic patients with a broad spectrum and severity of CHF. However, [Hb] was reduced and PV expanded in anaemic patients, highlighting haemodilution as an important factor contributing to low Hb in CHF. Abramov and colleagues [105] showed in a cohort of anaemic patients with low versus preserved left ventricular ejection fraction no correlation between [Hb] and RCV ($r = 0.09$, $p = 0.54$), although this was not the case in patients with normal blood volumes where [Hb] and RCV were significantly related to a moderate extent ($r = 0.55$, $p = 0.02$). The authors also elucidated that dilutional anaemia (PV expansion without a RCV deficit) was more common in patients with low versus preserved ventricular ejection fraction. In the study by Abramov and colleagues, RCV was not directly measured but derived from the quantification of PV, which has limitations as described previously in terms of measurement error, although

these may be small when comparing the validity between chromium and albumin dilution techniques [334]. I show a similar finding in the Chapter 5 whereby the strength of the relationship between [Hb] and tHb-mass varied with the extent of PV disturbance.

Decompensated CHF have also been studied in the context of volume status and anaemia. In a cohort of 32 decompensated chronic HF patients (with systolic dysfunction) hospitalised for management of symptomatic volume overload, Miller and colleagues [335] quantified PV directly using the radiolabelled albumin dilution technique with RCV subsequently derived. Distinct variability in RBC profiles were seen in the context of defining anaemia. Interestingly, 19 of the 32 patients met WHO criteria for anaemia. However, with the additional knowledge of RCV and PV, only 4/19 had a 'true' anaemia whereby both [Hb] and RCV were reduced. In contrast, 15/19 demonstrated an expanded PV (dilutional anaemia) with the remaining patients (13/32) having a normal [Hb] (12 of these patients actually had RCV excess).

Using the carbon monoxide rebreathing method, I extend such observations. To allow comparison I calculated RCV from BV and Hct, where BV is dependent on quantifying tHb-mass and [Hb]. I found a similar variability in RBC profiles in that 39 of the 109 participants were classified as anaemic based on WHO criteria, of which 15/39 had a RCV deficit and reduced [Hb]. However, only 2 (13.3 %) of these 15 patients had a RCV deficit, reduced [Hb] and normal PV (so called 'true' anaemia), with tHb-mass being 352 g and 449 g in these two patients. The remaining 13 patients were hypervolemic to varying extents (n= 5 mild to moderate, tHb-mass 515 ± 80 g, and n= 8 severe, tHb-mass 539 ± 105 g). Interestingly, 14 of the 39 anaemic patients had a relatively *raised* tHb-mass, with PV raised to a greater degree (n= 1 mild to moderate PV expansion, tHb-mass 610 g), n= 13 severe PV expansion, tHb-mass 758 ± 220 g. These data add further support to the argument that reliance on [Hb] can be misleading and provide a false view of a patients Hb status. Therefore, knowledge of tHb-mass, PV and [Hb] provides a more complete assessment of Hb and volume profiles in patients and allows volume excess and tHb-mass deficit to be defined separately and questions whether the concept of 'anaemia' needs readdressing.

5.5.1 Strengths and weaknesses

The strengths of this study include its prospective nature and use of the oCOR to quantify tHb-mass, as this shows comparable and likely lower measurement error compared to the gold standard ($^{51}\text{Chromium}$ radiolabelled RBCs) without any of the associated issues of handling radiolabelled blood. In addition, the oCOR method is of little inconvenience to patients, minimally invasive (requiring three blood samples from the earlobe, fingertip or cannula) and is safe, despite being performed on patients with serious medical conditions and comorbidities. I have shown that most patients can perform the technique, despite there being a degree of patient compliance with the rebreathing procedure like that required in more complex pulmonary function tests. This greatly widens the applicability of the oCOR test to measure tHb-mass and blood volume derivatives in the clinical setting, something I propose offers useful and additional information on the haematological status of patients compared to relying on more established and routine indices (e.g. [Hb], Hct) alone. It is acknowledged that it will not be possible or perhaps justified to implement the oCOR method to routine clinical practice. Instead, it may be an appropriate test in certain clinical contexts where further information on haematological and volume status is required. Indeed, certainly it would be justified to measure tHb-mass in cases where the aim of an intervention is to increase O_2 carrying capacity such as in anaemic cases following blood transfusion or iron supplementation.

Potential weaknesses of the current study include the relatively small sample size, although this study was adequately powered to explore the relationship between tHb-mass and [Hb] across sub-groups. The sample size of patients with chronic LD ($n=16$) is below the calculated 21 patients required based on the original *a priori* power calculation using the study by Hinrichs and colleagues [24] (see 5.3.6). This was related to difficulty recruiting sufficient patients and conflicting time restrictions in being able to test them, which although disappointing was out of my control. However, it is unlikely that the addition of 5 patients will have greatly altered the interpretation of results and subsequent narrative that tHb-mass is poorly related to [Hb] in CLD and CHF patients with PV being a key confounding factor.

The use of capillary and venous blood across testing sites (UCLH and Southampton) to quantify [Hb] is a weakness of this study, although blood sampling from venous, arterial and capillary blood have been shown to yield an identical $\Delta\%COHb$ and therefore identical tHb-mass values [272]. [Hb] measured in capillary blood has been widely shown to be higher [336-338] than [Hb] measured in venous blood. Such differences may be related to differences in body posture at the time of sampling, as well as factors affecting fingertip capillary samples such as skin thickness and temperature [339]. Biological variability may also in part explain differences in [Hb] values obtained from capillary and venous blood. All or some of these potential sources of error may have affected the measured [Hb] value and thus impacted upon the relationship between [Hb] and tHb-mass. To minimise such confounding factors, blood samples were taken by trained staff and [Hb] values obtained from capillary blood were corrected to venous conditions. In addition, all blood samples for the determination of [Hb], Hct and $\%COHb$ were collected under the same conditions, from the same anatomical site, with patients in a seated position and analysed within 10-15 minutes of one another due to $\%COHb$ being analysed after the oCOR test using a blood gas machine (which takes 15 minutes). The use of different blood gas machines and testing staff may have introduced error in the measurement of tHb-mass. However, I have shown a low typical error (TE) of repeat tHb-mass measurements in Chapter 4 of 1.93% (95% CI 1.3-3.4%) which is low and in keeping with other institutions [19, 285].

A method limitation in Chapter 5 (and Chapter 6) is that PV was not directly and independently measured, but was subsequently derived from BV once tHb-mass and [Hb] had been determined. Accordingly, PV values were dependent on the measurement of tHb-mass and [Hb]. Although the International Committee for Standardization in Haematology [116] suggest that human serum albumin labelled with iodine isotopes (^{131}I or ^{125}I) be used for the direct measurement of PV, in reality this was not feasible in this thesis given the time, costs and risks associated with techniques using radiolabelled isotopes, which precludes the widespread use of these techniques for routine and regular measurement of PV in the clinical setting. Perhaps future studies may wish to perform an independent assessment of PV in a representative subset of the total study population to determine the agreement between measured and

derived PV. Given that the primary aim of this Chapter was to explore the relationship between [Hb] and tHb-mass and that I have shown a low-test retest measurement error of the oCOR method in my hands to quantify tHb-mass, subsequently derived BV and PV may reflect this low level of error, although this cannot be confirmed in the current chapter.

5.6 Conclusion and further research

In conclusion, tHb-mass does not correlate with [Hb] in all patient groups and the relationship between tHb-mass and [Hb] appears to degrade as patients get sicker with PV a key confounding variable. There also appears to be distinct variability in RBC profiles of anaemic and non-anaemic patients when tHb-mass and PV are quantified in addition to [Hb]. Knowledge of tHb-mass, PV and [Hb] may provide a more comprehensive assessment of haematological and volume status compared to relying on [Hb] alone. This appears especially true amongst those in whom plasma volume may change most as a result of specific disease states.

Why may the findings from the current study be of clinical relevance? A low [Hb] is a trigger for a raft of further, perhaps unwarranted investigations (such as the assay of circulating haematinic factors) or treatments (such as the administration of packed red blood cells or iron supplementation), whilst denying the administration of agents to reduce plasma volume, which might sometimes be required. However, the inability to readily measure PV and tHb-mass means that patients are generally investigated or treated for a failure of Hb synthesis or of erythrocytosis. This may be inappropriate for a significant number of patients as I have shown in the current study. In other circumstances, contraction in plasma volume might offer false reassurance by maintaining [Hb], when tHb-mass is low. Indeed, measured [Hb], and the diagnosis of anaemia, can be strongly influenced by (or can largely depend upon) changes in plasma volume in so much that tHb-mass can be normal or elevated in anaemia. Therefore, if a reduced [Hb] is not a result of a 'true' deficit in tHb-mass, does this mean oxygen carrying capacity is impaired and that Hb optimisation is the appropriate treatment? In this case, a reduced [Hb]

may be the result of hypervolaemia (PV expansion) without any deficit in tHb-mass, and therefore alternative clinical management of volume status may be appropriate through diuretic therapy. I have shown in the current study that patients may have the same tHb-mass but vastly different [Hb], related to the extent of PV disturbance. Therefore, knowledge of tHb-mass, PV and [Hb] may provide a more comprehensive assessment of haematological status compared to relying on [Hb] alone and raises the question whether we need to readdress 'anaemia' as a concept in clinical practice and should we be defining volume excess and tHb-mass deficit separately instead?

I have demonstrated that the oCOR method is well tolerated by patients of varying ages with chronic diseases of mixed aetiology and severity. This, in addition to the previously highlighted advantages of using the oCOR technique over other methods greatly widens the potential applicability of using this technique in the clinical setting, which others may wish to adopt. Future studies may wish to investigate how best to normalise tHb-mass and derived blood volume compartments to allow adequate anthropometric reference values to be established. For example, body weight, lean body mass, body surface area or using allometric scaling techniques may be appropriate. In addition, the use of tHb-mass as a primary outcome when longitudinally monitoring the haematological status of patients following intervention is proposed given the larger variability in other haematological variables that I and others have shown. For example, using tHb-mass to assess the haematological status of patients throughout the perioperative period or quantifying changes in tHb-mass following iron supplementation or blood transfusion may be warranted.

Chapter 6

- 6 **Total haemoglobin mass is an important determinant of preoperative exercise capacity measured by cardiopulmonary exercise testing**

6.1 Abstract

Background. Impaired physical fitness, assessed by cardiopulmonary exercise testing (CPET) is associated with postoperative morbidity and mortality. Haemoglobin is the blood's oxygen-carrying pigment and anaemia, defined as a haemoglobin concentration ([Hb]) of $<130 \text{ g}\cdot\text{l}^{-1}$ in men and $<120 \text{ g}\cdot\text{l}^{-1}$ in women, is thus associated with such reduced fitness. However, anaemia may result not only from a fall in the absolute mass of haemoglobin (tHb-mass), but from a simple rise in the volume of plasma in which that haemoglobin is carried. Therefore, tHb-mass may correlate better with CPET-derived physical fitness in preoperative patients, than does [Hb]. I tested this hypothesis by exploring the relationship between [Hb], tHb-mass and preoperative CPET.

Methods. Patients awaiting major elective surgery underwent CPET. tHb-mass was quantified using the optimised carbon-monoxide (oCOR) rebreathing method and circulating [Hb] determined.

Results. Forty-two patients (83% male) were enrolled and successfully completed CPET and the oCOR test, of which 26% were anaemic. Mean \pm SD peak oxygen consumption ($\dot{V}\text{O}_2$ peak) and oxygen consumption at anaerobic threshold ($\dot{V}\text{O}_2$ at AT- the point at which anaerobic metabolism significantly supplements aerobic metabolism) were 15.7 ± 5.9 and $10.2 \pm 2.3 \text{ ml}\cdot\text{kg}^{-1}\text{min}^{-1}$, respectively. Haemoglobin concentration was unrelated to $\dot{V}\text{O}_2$ at AT ($r = 0.023$, $p = 0.892$) or $\dot{V}\text{O}_2$ peak ($r = 0.041$, $p = 0.796$) expressed in $\text{ml}\cdot\text{kg}^{-1}\text{min}^{-1}$. In contrast, tHb-mass ($\text{g}\cdot\text{kg}^{-1}$) was related to $\dot{V}\text{O}_2$ peak ($r = 0.483$, $p = 0.001$), and more so to $\dot{V}\text{O}_2$ at AT ($r = 0.661$, $p < 0.0001$) expressed in $\text{ml}\cdot\text{kg}^{-1}\text{min}^{-1}$. tHb-mass ($\text{g}\cdot\text{kg}^{-1}$) explained 43.7% of $\dot{V}\text{O}_2$ at AT ($p < 0.0001$) and 23.3% of $\dot{V}\text{O}_2$ peak ($p = 0.001$) $\text{ml}\cdot\text{kg}^{-1}\text{min}^{-1}$. [Hb] did not explain any variance in either measure. A $1 \text{ g}\cdot\text{kg}^{-1}$ increase in tHb-mass was associated with a $1.0 \text{ ml}\cdot\text{kg}^{-1}\text{min}^{-1}$ increase in AT ($p < 0.0001$) and a $2.0 \text{ ml}\cdot\text{kg}^{-1}\text{min}^{-1}$ increase in $\dot{V}\text{O}_2$ peak ($p = 0.01$), after adjusting for age, sex, smoking status, diabetes and the presence of cardiovascular disease.

Conclusions. Unlike [Hb], tHb-mass is an important determinant of preoperative patient physical fitness associated with exertional oxygen consumption. It is both measurable and manipulable (through targeted transfusion of red blood

cells) and therefore trials of targeted increases in tHb-mass to enhance physical fitness and improve surgical outcome thus appear warranted.

6.2 Introduction

Cardiopulmonary exercise testing (CPET) measures the integrated function of the cardiovascular, pulmonary and musculoskeletal systems and provides an objective assessment of physical fitness. CPET is routinely used for the purposes of preoperative assessment and risk stratification prior to major surgery and to guide care postoperatively [193, 194] with the aim of improving patient outcomes. CPET measures exertional oxygen consumption ($\dot{V}O_2$) at peak exercise (the peak exertional oxygen consumption, $\dot{V}O_2$ peak), and at the anaerobic threshold ($\dot{V}O_2$ at AT), being a submaximal marker of physiological reserve. When reduced, $\dot{V}O_2$ peak and $\dot{V}O_2$ at AT are associated with an increased risk of morbidity and mortality [56, 58, 195] following major surgery.

Haemoglobin (Hb) is the blood's O_2 carrying pigment carried in circulating red blood cells (RBCs). Accordingly, the availability of Hb may influence cardiorespiratory fitness. Preoperative anaemia is defined using whole blood as a circulating haemoglobin concentration ($[Hb]$) $<130\text{ g}\cdot\text{l}^{-1}$ in men and $<120\text{ g}\cdot\text{l}^{-1}$ in women [2]. Preoperative anaemia is common and affects around one third of cases [6], is associated with decreased exercise capacity [11, 60] and an increased incidence of postoperative morbidity and mortality [6, 13]. However, because the measured $[Hb]$ is based on whole blood it reflects both the mass of circulating Hb (tHb-mass), and the volume status of patients, specifically the volume of plasma in which erythrocytes are suspended. The 2-minute optimised carbon monoxide rebreathing (oCOR) method developed by Schmidt and Prommer [19] provides a precise, safe and reliable technique to quantify tHb-mass, although this method has rarely been applied in the clinical setting despite its many advantages over previous techniques.

Because the proportion of O_2 carried in plasma is trivial ($0.3\text{ ml } O_2$ per 100 ml^{-1} blood), the relationship between exercise capacity and tHb-mass might be stronger than that with $[Hb]$. Indeed, a close relationship between tHb-mass and

aerobic capacity has been demonstrated in healthy volunteers and athletes [250, 340] but whether this applies to patient populations is less certain. Indeed, no studies have measured tHb-mass in patients awaiting major surgery and investigated associations with CPET-derived exertional oxygen consumption. In Chapter 6 I tested this hypothesis.

6.2.1 Study aim

To explore the relationship between both [Hb] and tHb-mass and measures of patient physical fitness, determined by cardiopulmonary exercise testing (CPET).

6.2.2 Study hypothesis

tHb-mass will display a stronger relationship with exertional $\dot{V}O_2$ at AT and $\dot{V}O_{2\text{peak}}$ than [Hb].

6.3 Methods

Ethical approval was granted by the London, Camden and Kings Cross Research Ethics Committee (REC reference: 13/LO/1901). We fully adhered to Caldicott guidelines and conformed to the standards set by the Declaration of Helsinki. Patients were given a participant information sheet (see Appendix E) prior to written informed consent being obtained from all participants willing to participate (see Appendix F).

6.3.1 Patients

Adult (> 18 years) elective (non-cardiac) surgical patients at University College Hospital (University College London Hospitals NHS Foundation Trust) and Southampton General Hospital (University Hospital Southampton NHS Foundation Trust) were prospectively studied between February and August 2015. Patients were either receiving CPET as part of their routine preoperative assessment, or were co-recruits to the 'METS' study of the role of CPET in altering surgical outcome [341]. Patients were sent a participant information sheet by a member the CPET team enclosed with their routine CPET appointment letter. Subsequently, patients were contacted regarding their voluntary participation in the study. Age, gender, height, weight, diagnosis, planned surgical procedure, comorbidities (such as diagnosis of diabetes, respiratory or cardiovascular disease) and current medications were documented.

6.3.1.1 Inclusion criteria

- i) Male or female adults (> 18 years)
- ii) Able to perform CPET
- iii) Able to perform oCOR technique
- iv) Able to consent
- v) Awaiting major surgery

6.3.1.2 Exclusion criteria

- i) Adults with known underlying history of learning disabilities, or adults who do not have mental capacity to consent for themselves.
- ii) Prisoners.
- iii) Have contraindication to CPET as outlined in ATS/ACCP guidelines.
- iv) Baseline %COHb greater than 5% (as commonly occurs in smokers).
- v) Haemoglobinopathies (e.g. Sickle cell anaemia).

6.3.2 Cardiopulmonary Exercise Testing

CPET was performed according to the established and widely used American Thoracic Society/American College of Chest Physicians (ATS/ACCP) guidelines on CPET [274], see methods section 3.2 for full details.

6.3.3 Statistics

Statistical analysis was performed using SPSS Statistics (Version 23.0 for Apple Macintosh, Chicago, IL, USA). Values are presented as mean \pm standard deviation (SD), unless otherwise stated. Median and interquartile range (IQR) are reported when variables are not normally distributed and when data transformation (logarithmic, square root or reciprocal) did not result in a normally distributed variable. Categorical variables are presented as frequency (%). Normal (Gaussian) distribution was assessed using a combination of the Kolmogorov-Smirnov test, visual inspection of histogram charts and normal Q-Q plots for each variable. Independent samples t-test and Mann-Whitney U tests assessed the differences in haematological variables in anaemic and non-anaemic patients, depending on normality of data distribution. Pearson's correlation coefficient assessed the relationship between [Hb], tHb-mass and exertional $\dot{V}O_2$ allowing adjustment for confounding. Linear regression models assessed the associations between $\dot{V}O_2$ and haematological variables ([Hb] and tHb-mass) and the proportion of variance in $\dot{V}O_2$ explained by [Hb] and tHb-mass, allowing adjustment for confounding. Specifically, age, gender,

smoking status, diabetes and the presence of cardiovascular disease (defined if a patient had either ischaemic heart disease, heart failure or previous stroke) were included as confounders in both correlation and regression analyses. [Hb], tHb-mass, exertional $\dot{V}O_2$ at AT and $\dot{V}O_2$ peak were expressed in $g \cdot l^{-1}$, $g \cdot kg^{-1}$ and $ml \cdot kg^{-1} \cdot min^{-1}$, respectively for both correlation and regression analysis. Elsewhere, $\dot{V}O_2$ is expressed in $ml \cdot kg^{-1} \cdot min^{-1}$ and tHb-mass in $g \cdot kg^{-1}$ unless otherwise stated. All tests were two-sided with statistical significance accepted as a p-value of < 0.05 .

6.3.4 Power calculation

The original *a priori* power calculation for this study was performed to be able to detect significant differences in both exertional $\dot{V}O_2$ and tHb-mass between patients with and without postoperative morbidity on day-5. Specifically, using data from West and colleagues [342] I anticipated that 50% of patients would have a post-operative morbidity by day-5 and the standard deviation of $\dot{V}O_2$ at AT to be $3.35 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. For tHb-mass, based on the study by Koponen and colleagues [49] I assumed that the standard deviation of tHb-mass would be $1.25 \text{ g} \cdot \text{kg}^{-1}$. Allowing for a 10% loss to follow-up, 91 patients would provide 80% power at the 5% significance level to detect a minimum, clinically important difference in mean $\dot{V}O_2$ at AT of $2.1 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ and a difference in mean total haemoglobin mass of $0.78 \text{ g} \cdot \text{kg}^{-1}$ between those patients with and without day-5 postoperative morbidity.

However, during patient recruitment and data collection it became clear that it was not going to be possible to recruit the 91 patients required to meet the original power calculation to look at postoperative outcome in this study. The main hypothesis of this study was that tHb-mass would display a stronger relationship with exertional $\dot{V}O_2$ than [Hb] and therefore to quantify these relationships was the primary aim of this study and while it would have been of interest to investigate links to postoperative outcome, at this stage it was not possible nor was it the primary aim. Subsequently I performed a *post hoc* power calculation using G*3 Power version 3.1.9.2 [316] to establish an achieved power for this study of 0.91, based on the sample size of 42 patients,

an alpha value of 0.05 and observed correlation of $r=0.48$ between weight adjusted tHb-mass and $\dot{V}O_2$ peak ($\text{ml}\cdot\text{kg}^{-1}\text{min}^{-1}$).

6.4 Results

Forty-three patients (19 from Southampton General Hospital and 24 from University College London Hospital) consented to take part in the study, of whom 10 were co-recruits of the 'METS' study participants [341]. One failed to successfully complete the oCOR protocol and so was excluded. The patient characteristics, including surgical specialty are shown in Table 6-3.

No major adverse clinical events occurred during CPET that resulted in premature exercise termination. The mean \pm SD $\dot{V}O_2$ peak and AT were 15.7 ± 5.9 and $10.2 \pm 2.3 \text{ ml}\cdot\text{kg}^{-1}\text{min}^{-1}$. AT was unable to be determined in 3 patients but these remain in any analysis containing $\dot{V}O_2$ peak. Mean \pm SD [Hb] and tHb-mass were $135.6 \pm 15.6 \text{ g}\cdot\text{l}^{-1}$ and $677 \pm 146 \text{ g}$, respectively. Table 6-4 shows haematological variables for all patients and categorised by anaemia classification.

6.4.1 Anaemia and haematological variables

Twenty-six percent of patients were anaemic preoperatively per WHO criteria. Thus, anaemic patients had lower [Hb] and Hct compared to non-anaemic patients (see Table 6-4). There was no difference in tHb-mass between groups when expressed either in absolute grams of haemoglobin or when scaled to body mass (kg). Anaemic patients displayed an expanded blood volume ($\text{ml}\cdot\text{kg}^{-1}$) and plasma volume (ml & $\text{ml}\cdot\text{kg}^{-1}$) compared to non-anaemic patients (see Figure 6-1 B and D, respectively).

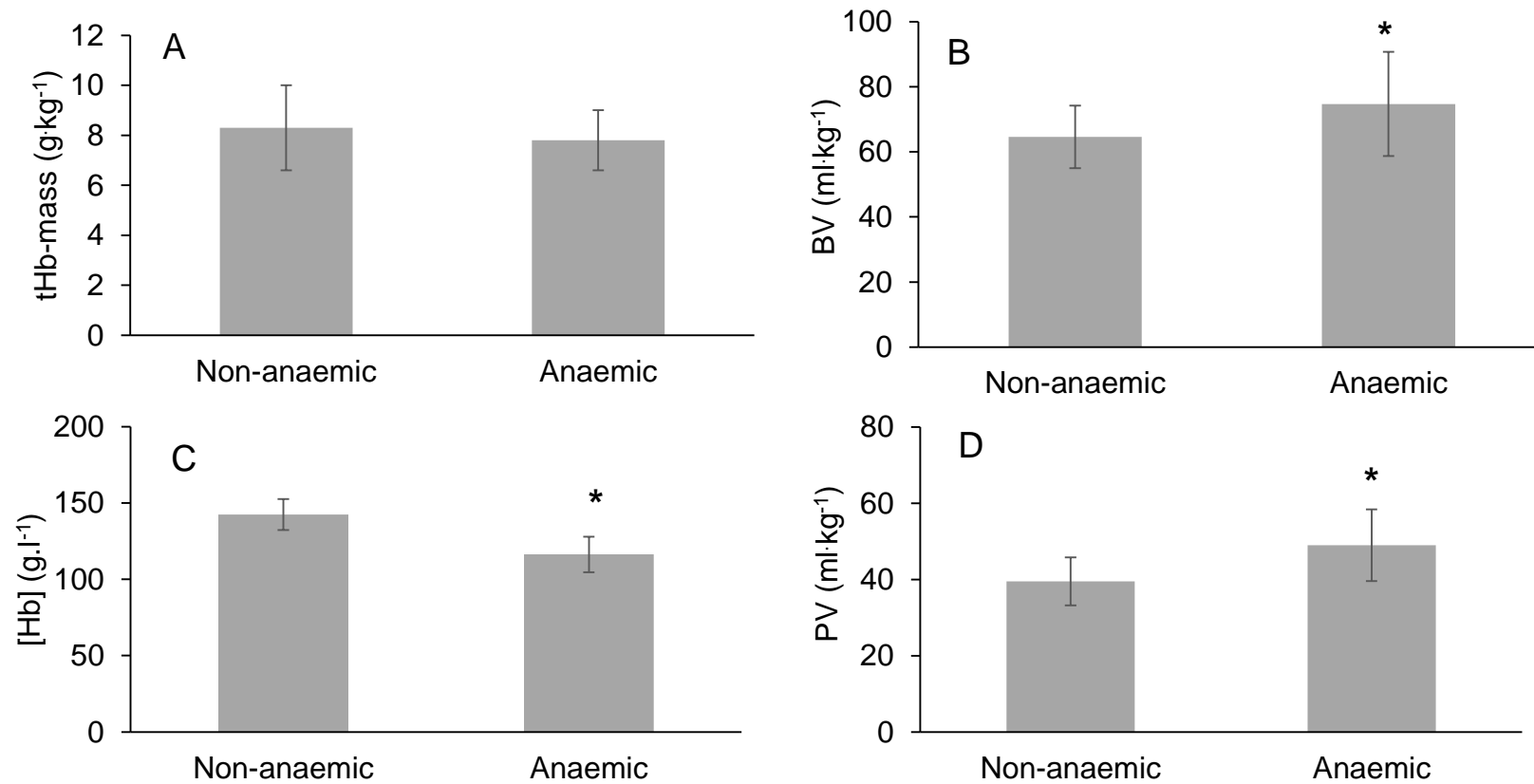


Figure 6-1. (A) Total haemoglobin mass (tHb-mass), (B) blood volume (BV), (C) haemoglobin concentration ([Hb]), (D) plasma volume (PV) in anaemic and non-anaemic CPET patients. * $p < 0.05$

6.4.2 Relationships between haematological variables and oxygen uptake

[Hb] ($\text{g}\cdot\text{l}^{-1}$) was unrelated to either $\dot{V}\text{O}_2$ at AT ($r = 0.023$, $p = 0.892$) or $\dot{V}\text{O}_2$ peak ($r = 0.041$, $p = 0.796$) expressed in $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. However, tHb-mass ($\text{g}\cdot\text{kg}^{-1}$) showed a moderate association with $\dot{V}\text{O}_2$ peak, ($r = 0.483$, $p = 0.001$) and, moreover, with $\dot{V}\text{O}_2$ at AT ($r = 0.661$, $p < 0.0001$), expressed in $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (see Figure 6-2). The relationships between tHb-mass (g) and $\dot{V}\text{O}_2$ at AT ($\text{ml}\cdot\text{min}^{-1}$) was $r = 0.700$, $p < 0.0001$ and between tHb-mass (g) and $\dot{V}\text{O}_2$ peak ($\text{ml}\cdot\text{min}^{-1}$), $r = 0.522$, $p < 0.0001$. After adjusting for age, gender, diabetes, smoking status, and the presence of cardiovascular disease, tHb-mass ($\text{g}\cdot\text{kg}^{-1}$) remained strongly correlated with $\dot{V}\text{O}_2$ at AT ($r = 0.629$, $p < 0.0001$) and to a lesser extent with $\dot{V}\text{O}_2$ peak ($r = 0.412$, $p = 0.011$) expressed in $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$.

6.4.3 Linear Regression Models

[Hb] failed to significantly explain any of the variance in $\dot{V}\text{O}_2$ at AT (adjusted $R^2 = -0.027$, $p = 0.892$) or $\dot{V}\text{O}_2$ peak (adjusted $R^2 = -0.023$, $p = 0.796$) expressed in $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. In contrast, tHb-mass ($\text{g}\cdot\text{kg}^{-1}$) explained 43.7% of the variance in $\dot{V}\text{O}_2$ at AT ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) $p < 0.0001$ and 23.3% in $\dot{V}\text{O}_2$ peak ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) $p = 0.001$. After adjusting for age, gender, smoking status, diabetes, and the presence of cardiovascular disease, only tHb-mass ($\text{g}\cdot\text{kg}^{-1}$) was associated with $\dot{V}\text{O}_2$ at AT ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) with a 1 $\text{g}\cdot\text{kg}^{-1}$ increase in tHb-mass associated with a 1.0 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ increase in AT ($p < 0.0001$). Similarly, after adjustment for age, gender, smoking status, diabetes, and the presence of cardiovascular disease, only tHb-mass ($\text{g}\cdot\text{kg}^{-1}$) was associated with $\dot{V}\text{O}_2$ peak ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) with a 1 $\text{g}\cdot\text{kg}^{-1}$ increase in tHb-mass associated with a 2.0 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ increase in $\dot{V}\text{O}_2$ peak ($p = 0.01$). See Table 6-1 and Table 6-2 for multiple linear regression coefficients for factors assessed in the prediction of $\dot{V}\text{O}_2$ at AT and $\dot{V}\text{O}_2$ peak, respectively.

Table 6-1. Multiple linear regression coefficients for factors assessed in the prediction of oxygen consumption at anaerobic threshold ($\dot{V}O_2$ AT, $\text{ml}\cdot\text{kg}^{-1}\text{ min}^{-1}$)

	β	SE β	Standardised β	Sig
Constant	1.95	3.39		0.569
tHb-mass	1.09	0.24	0.64	<0.0001
Age	-0.02	0.03	-0.09	0.480
Gender	0.98	0.84	0.16	0.252
Diabetes	-0.17	0.72	-0.03	0.811
Smoking status	-0.42	0.40	-0.13	0.300
Cardiovascular disease	-1.41	0.88	-0.21	0.121

SE, standard error; tHb-mass (total haemoglobin mass, $\text{g}\cdot\text{kg}^{-1}$), age (yr), gender (male or female), cardiovascular disease (presence of either ischaemic heart disease, chronic heart failure or stroke), smoking status (current, former or non-smoker), diabetes (present of absent).

Table 6-2. Multiple linear regression coefficients for factors assessed in the prediction of peak oxygen consumption ($\dot{V}O_2$ peak, $\text{ml}\cdot\text{kg}^{-1}\text{ min}^{-1}$)

	β	SE β	Standardised β	Sig
Constant	4.86	10.20		0.636
tHb-mass	1.94	0.73	0.44	0.011
Age	-0.07	0.08	-0.13	0.353
Gender	0.96	2.46	0.06	0.699
Diabetes	0.57	2.16	0.04	0.791
Smoking status	-1.84	1.15	-0.23	0.117
Cardiovascular disease	-2.66	2.49	-0.16	0.292

SE, standard error; tHb-mass (total haemoglobin mass, $\text{g}\cdot\text{kg}^{-1}$), age (yr), gender (male or female), cardiovascular disease (presence of either ischaemic heart disease, chronic heart failure or stroke), smoking status (current, former or non-smoker), diabetes (present of absent).

Table 6-3. Patient characteristics and medications.

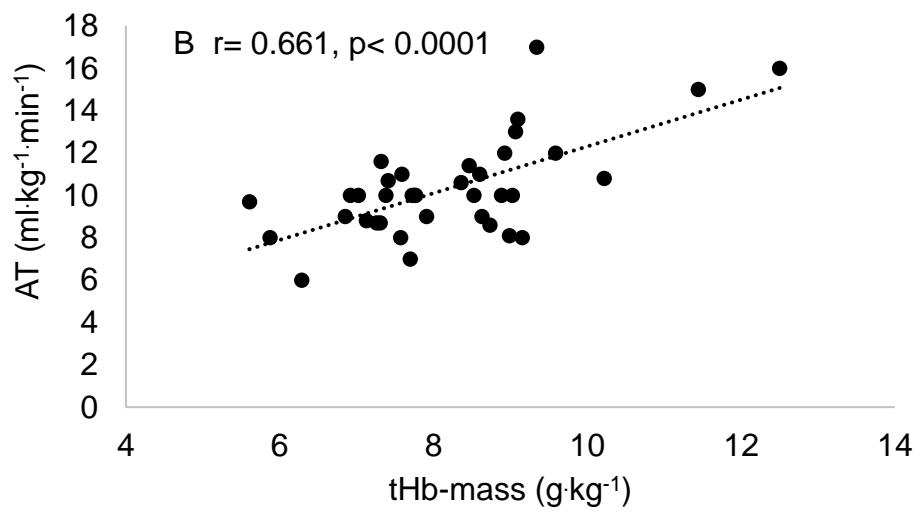
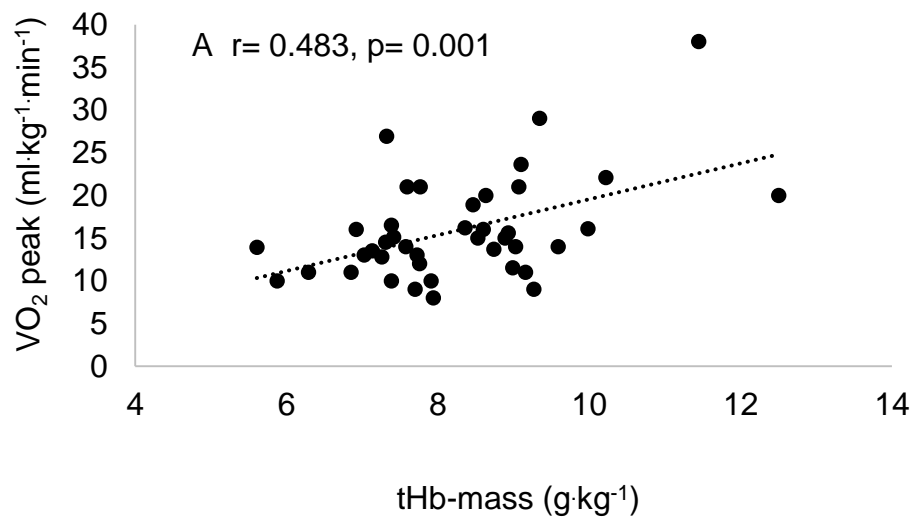
Variable	n= 42
<i>Gender</i>	
Male	35 (83%)
Female	7 (17%)
Age (yr)	66 (58-71)
Height (cm)	172 ± 9
Weight (kg)	83 ± 16
<i>Surgical specialty</i>	
Urology	19
Hepatology	9
Maxillofacial	2
Upper gastrointestinal	3
Thoracic	4
Vascular	1
Gastrointestinal	4
Ischaemic heart disease	5 (12%)
Heart failure	1 (2%)
Hypertension	19 (45%)
Stroke	1 (2%)
Diabetes	9 (21%)
COPD	6 (14%)
Smoking history	
Current	6 (14%)
Former	12 (27%)
Never	24 (57%)
<i>Medication</i>	
Beta blocker	6 (14%)
Nitrate therapy	1 (2%)
ACE inhibitor	14 (33%)
Statin	13 (31%)

COPD, chronic obstructive pulmonary disease; ACE inhibitor, angiotensin converting-enzyme inhibitor. Values are expressed as mean ± SD, median (IQR) or frequency (%).

Table 6-4. Haematological characteristics in anaemic and non-anaemic CPET patients.

Variable	All patients (n= 42)	Anaemic (n= 11)	Non-anaemic (n= 31)	p-value
[Hb] (g·l ⁻¹)	135.6 ±15.6	116.3 ± 11.7	142.5 ± 10.1	< 0.0001
Hct (%)	41.4 ± 4.0	37.4 ± 4.2	42.7 ± 3.0	< 0.0001
tHb-mass (g)	677 ± 146	610 ±119	700 ± 149	0.079
tHb-mass (g·kg ⁻¹)	8.2 ± 1.3	7.8 ± 1.7	8.3 ± 1.2	0.316
BV (ml)	5495 ± 1065	5753 ± 951	5404 ± 1102	0.356
BV (ml·kg ⁻¹)	67.3 ± 12.2	74.7 ± 16.0	64.6 ± 9.6	0.016
PV (ml)	3421 ± 678	3775 ± 560	3295 ± 680	0.043
PV (ml·kg ⁻¹)	42.0 ± 8.2	49.0 ± 9.4	39.5 ± 6.3	0.001
PV (%)	62.3 ± 3.7	65.9 ± 3.8	61.0 ± 2.7	< 0.0001
RCV (ml)	2074 ± 462	1978 ± 466	2108 ± 464	0.431
RCV (ml·kg ⁻¹)	25.3 ± 4.9	25.6 ± 7.4	25.1 ± 3.9	0.768
MCV (fl)	90.5 ± 4.6	90.6 ± 4.3	89.4 ± 5.0	0.521
MCH (pg)	30.3 ± 1.9	29.7 ±1.4	30.4 ± 2.1	0.370
MCHC (g·l ⁻¹)	342 (321-349)	320 (314-347)	344 (331-350)	0.096
Creatinine (μmol·l ⁻¹)	97 (65-102)	112 (67-188)	77 (65-97)	0.107
Albumin (g·l ⁻¹)	42 (39-44)	36.0 (27-43)	43 (40-45)	0.042

[Hb], Haemoglobin concentration; Hct, haematocrit; tHb-mass, total haemoglobin mass; BV, blood volume; PV, plasma volume; RCV, red cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration. Anaemia defined per World Health Organisation criteria when ([Hb]) <130 g·l⁻¹ in men and <120 g·l⁻¹ in women. Values are expressed as mean ± SD, median (interquartile range, IQR) or frequency (%).



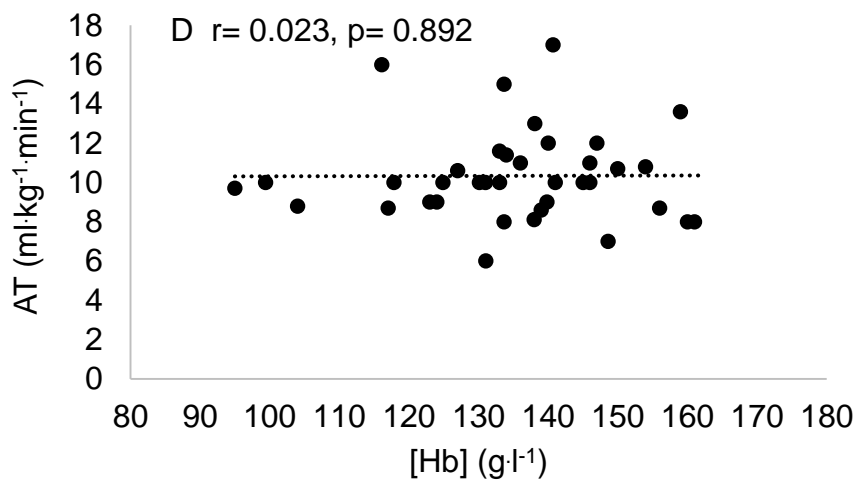
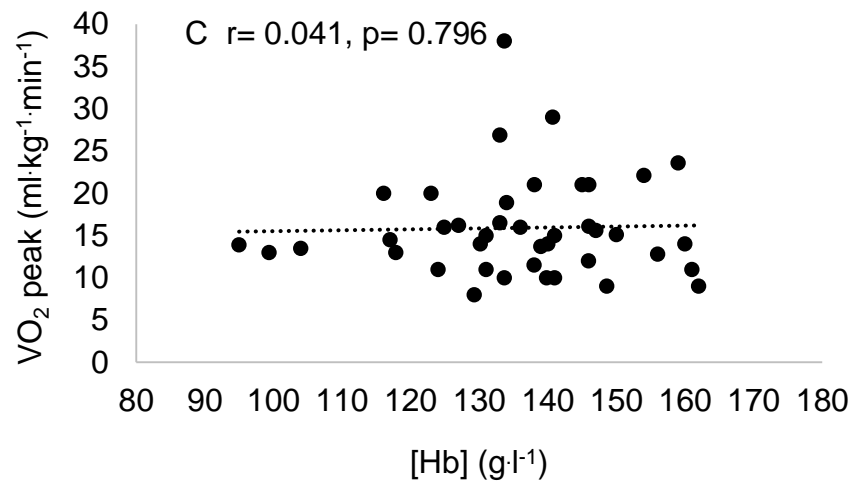


Figure 6-2. (A) Unadjusted relationship between haematological variables and exertional oxygen consumption. Relation of $\dot{V}O_2$ peak (ml·kg⁻¹·min⁻¹) to tHb-mass (g·kg⁻¹) (A) and [Hb] (g·l⁻¹) (C) in 42 patients. Relation of $\dot{V}O_2$ at AT to tHb-mass (g·kg⁻¹) (B) and [Hb] (g·l⁻¹) (D) in 39 patients. tHb-mass, total haemoglobin mass; [Hb], haemoglobin concentration; AT, anaerobic threshold; $\dot{V}O_2$ peak, peak oxygen consumption.

6.5 Discussion

To my knowledge this is the only study to have explored the relationship between total haemoglobin mass and physical fitness determined by cardiopulmonary exercise testing in patients awaiting major elective surgery. Whilst [Hb] was unrelated to exertional $\dot{V}O_2$ at either AT or $\dot{V}O_2$ peak ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), total haemoglobin mass ($\text{g}\cdot\text{kg}^{-1}$) was associated with $\dot{V}O_2$ peak ($r=0.48$) and to a greater extent with $\dot{V}O_2$ at AT ($r=0.66$), even after adjusting for measured confounding variables. These relationships strengthened when comparing absolute tHb-mass (g) with $\dot{V}O_2$ at AT ($\text{ml}\cdot\text{min}^{-1}$) ($r=0.700$ $p<0.0001$) and $\dot{V}O_2$ peak ($\text{ml}\cdot\text{min}^{-1}$), $r=0.522$, $p<0.0001$. Some 44% of the variance in a patient's $\dot{V}O_2$ at AT ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) was attributable to measured tHb-mass ($\text{g}\cdot\text{kg}^{-1}$), which may be of particular importance in the perioperative setting, given the consistent link between $\dot{V}O_2$ at AT and surgical outcome.

There is limited research in the clinical setting with which to compare my results. However, one study by Koponen and colleagues [49] measured total haemoglobin mass using the oCOR method and aerobic capacity ($\dot{V}O_2$ peak) quantified by CPET in 12 men with type 1 diabetes (T1DM) and healthy controls ($n=23$). [Hb] was not associated with $\dot{V}O_2$ peak either in patients with T1DM or controls, in keeping with results from the current study. In patients with T1DM, body mass-normalised tHb-mass was strongly related with $\dot{V}O_2$ peak ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), $r=0.71$; $p<0.01$ and tHb-mass explained 51% of the variance in $\dot{V}O_2$ peak. This study did not report the relationship between tHb-mass and AT however. The strength of correlation between tHb-mass ($\text{g}\cdot\text{kg}^{-1}$) and $\dot{V}O_2$ peak ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), $r=0.71$ reported by Koponen and colleagues [49] is stronger than results from the current study ($r=0.48$). This may in part be explained by differences in relatively small sample sizes, differences (albeit small) in measurement error inherent in both oCOR test and CPET measurement and perhaps to a greater extent related to $\dot{V}O_2$ peak being dependent upon patient/participant volition and motivation. If this is the case then the relationships between $\dot{V}O_2$ peak and tHb-mass may be underestimated in the current study. This is one proposed advantage of using the AT to quantify

preoperative fitness as it is a non-volitional measure of physical fitness. In general, the finding that tHb-mass displays a stronger relationship with $\dot{V}O_2$ peak than [Hb] is consistent with previous reports in healthy volunteers and trained athletes ($r = 0.79$ in pooled data from 611 subjects, [340]; $r = 0.48, 0.79$ and 0.92 in male runners, and male and female rowers, respectively [29]).

Mean AT of $10 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and $\dot{V}O_2$ peak of $16 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ are in keeping with previous studies quantifying preoperative exercise capacity using CPET ([58, 59]). Similarly, the prevalence of anaemia (26%) in the current study is in keeping with previously reported values of around 30% from large, retrospective datasets ([6, 13]), supporting anaemia as a common occurrence in patients awaiting major surgery. The mean \pm SD tHb-mass (g and $\text{g}\cdot\text{kg}^{-1}$) from the current study were $677 \pm 146 \text{ g}$ and $8.2 \pm 1.3 \text{ g}\cdot\text{kg}^{-1}$, respectively. These values are lower than those report by Koponen and colleagues [49] (tHb-mass $722 \pm 121 \text{ g}$ and $10.1 \pm 1.5 \text{ g}\cdot\text{kg}^{-1}$) in patients with T1DM and healthy controls (tHb-mass $898 \pm 96 \text{ g}$ and $11.0 \pm 1.1 \text{ g}\cdot\text{kg}^{-1}$). Furthermore, values reported in the current study are substantially lower than those of endurance trained athletes (mean \pm SD tHb-mass $1285 \pm 123 \text{ g}$ and $13.7 \pm 0.5 \text{ g}\cdot\text{kg}^{-1}$) in elite rowers, [25]; tHb-mass $958 \pm 123 \text{ g}$ in trained cyclists, [27] and in excess of $14.5 \text{ g}\cdot\text{kg}^{-1}$ in elite marathon runners [261].

Data from endurance-trained athletes suggest that such high tHb-mass values can only be reached by either years of intensive training or are strongly influenced by genetic factors [209], but may also be influenced by elicited use of erythropoietin and/or blood transfusion, previously common practice in many endurance sports. However, data from untrained subjects also suggests that improvements in $\dot{V}O_2$ peak following only 6 weeks of endurance training can also be achieved, with increases in peak \dot{Q} and O_2 carrying capacity (quantified by RCV and tHb-mass) being the primary underlying mechanisms [344]. Indeed, as Schmidt and Prommer hypothesise [209], tHb-mass governs aerobic exercise capacity via two mechanisms through which $\dot{V}O_{2\text{max}}$ can be enhanced as a result of i) a balanced increase in both tHb-mass and PV (thus increasing \dot{Q}) and ii) by elevating [Hb] in the context of reduced or unaltered PV, which augments the arteriovenous oxygen content difference (oxygen extraction). It is

this dual role which may explain the observed stronger relationship between tHb-mass and exertional $\dot{V}O_2$ in the current study.

Given that prolonged endurance training is not feasible in patients awaiting major surgery, and genetics are predetermined, alternative interventions to increase tHb-mass preoperatively (and thus aerobic fitness and related surgical outcome) are worthy of exploration. In addition, preoperative anaemia is associated with an increased risk of postoperative morbidity and mortality [6, 13] and it would thus perhaps be surprising if a similar relationship was lacking for tHb-mass. Furthermore, as I have shown a close relationship between tHb-mass and preoperative fitness, and given the link between exertional $\dot{V}O_2$ and surgical outcome, it would seem logical to purport that a high tHb-mass may be advantageous to patient outcomes in the perioperative setting, although this remains to be confirmed. It should be highlighted that aerobic exercise training causes many other important physiological adaptations, such as altered cardiac structure and function [344] and changes within skeletal muscle [345, 346] that may lead to enhanced functional capacity in patients independently of changes in tHb-mass, with even small gains potentially of clinical significance, particularly in the perioperative period. Indeed, this is an area of growing research in patients awaiting major surgery to optimise physical fitness preoperatively (termed prehabilitation), with the aim of improving postoperative outcome. Optimisation of tHb-mass may also have a role to play in prehabilitation.

The underlying causes of exercise intolerance are multifactorial, especially in patients with multiple co-morbidities commonly encountered preoperatively. However, and although subject to much debate [229], it is widely considered that reduced $\dot{V}O_2$ peak and $\dot{V}O_2$ at AT are related to an impairment in systemic D_{O_2} relative to metabolic demands [230, 277], although muscle fibre type recruitment and intramuscular enzymatic rate limitations have also been proposed as important limiting factors [347, 348]. Given that D_{O_2} is dependent on \dot{Q} and arterial oxygen content, and that many patients are anaemic preoperatively (or at least may have lower haemoglobin values) this adds weight to the argument that increasing tHb-mass should be favourable as

purported above. Certainly, the relationship of tHb-mass with exertional $\dot{V}O_2$ at AT and $\dot{V}O_2$ peak in Chapter 6 would hint towards this mechanism.

Once tHb-mass has been quantified, other blood volume compartments (BV, RCV and PV) can be derived providing [Hb] and Hct (%) are measured. In the context of anaemia, as I have also shown in Chapter 5, these variables offer additional insight into the haematological and volume status of patients given that the measured [Hb] can be affected by both changes in tHb-mass and PV, independently of one another. As shown in Table 6-4, tHb-mass (g & $g \cdot kg^{-1}$) and RCV (ml & $ml \cdot kg^{-1}$) did not statistically significantly differ between anaemic and non-anaemic patients. However, BV ($ml \cdot kg^{-1}$) and PV (ml & $ml \cdot kg^{-1}$) were expanded in anaemic patients, suggesting to some extent that anaemia in this small sample may in part be related to PV expansion rather than a deficit in O_2 carrying capacity as quantified by tHb-mass. Thus, dilutional anaemia may not have such negative effects on exercise capacity as 'true' anaemia, being characterised by a deficit in tHb-mass and impaired blood O_2 carriage. This may to some extent explain the lack of association between [Hb] and exertional $\dot{V}O_2$ I found in the current chapter. In future studies the aetiology of anaemia should therefore also be evaluated. Indeed, in the current study median creatinine concentrations were numerically (but not statistically significantly) higher in anaemic patients, suggesting chronic kidney disease as a potential underlying cause of anaemia. However, it was not the aim of this study to elucidate the underlying aetiology of anaemia, and larger studies (with greater power) would be needed to determine if renal impairment contributed significantly to the prevalence of preoperative anaemia.

6.5.1 Strengths and weaknesses

Strengths of this study include its use of CPET to prospectively determine preoperative exercise capacity as it is considered the gold standard assessment of cardiorespiratory fitness, being both objective and precise. As described previously in Chapter 5, the use of the oCOR method to quantify tHb-mass is a strength. Potential weaknesses of the current study include the relatively small sample size, although this study was adequately powered to

explore the relationship between tHb-mass and exertional $\dot{V}O_2$ (*post hoc* achieved power of 0.91, based on the sample size of 42, alpha value of 0.05 and correlation of $r=0.48$ between weight adjusted tHb-mass and $\dot{V}O_2$ peak). In addition, to my knowledge, the current study cohort is the largest in which the relationship between preoperative tHb-mass and CPET-derived physical fitness has been explored and is a larger sample size than the study by Koponen and colleagues [49]. As outlined in detail in Chapter 5 the use of capillary and venous blood across testing sites (UCLH and Southampton) to quantify [Hb] is a weakness of this study as is the use of different blood gas machines between sites. I have already addressed in Chapter 5 how I minimised and/or controlled for these factors. Finally, the use of different metabolic carts across sites may have impacted upon measured $\dot{V}O_2$, although both metabolic carts were calibrated prior to every CPET to reduce any measurement error in exertional $\dot{V}O_2$.

6.6 Conclusion and further research

In conclusion, in a cohort of patients awaiting major elective surgery, total haemoglobin mass was found to be an important determinant of preoperative cardiorespiratory fitness associated with exertional oxygen consumption, whereas haemoglobin concentration failed to show any significant relationship with exertional $\dot{V}O_2$. Future studies may wish to address whether increasing tHb-mass preoperatively leads to improved fitness for surgery as quantified by CPET. Subsequently, adequately powered and randomised controlled trials will be required to see if manipulation of tHb-mass affects surgical outcome, which the current Chapter 6 was not designed or powered to address.

7 Overall conclusion and future directions

In this section I provide a general conclusion to my thesis and provide possible areas for future research based on my work to date. At the end of this section I also provide a detailed abstract style overview for four future experiments.

In this thesis, I have utilised the oCOR method to quantify tHb-mass in different patient populations and disease states and objectively measured cardiorespiratory fitness in patients awaiting surgery using the gold standard test, CPET. In Chapter 4 I have shown that the oCOR method is reliable in my hands based on a low-test retest measurement error that is similar to other established institutions using the oCOR method. In addition, the oCOR is well tolerated by patients of varying ages with chronic diseases of mixed aetiology and severity, is safe and minimally invasive. This greatly widens the potential applicability of the oCOR test to measure tHb-mass and blood volume derivatives more routinely in the clinical setting, something I propose offers useful and additional information on the haematological and volume status of patients compared to relying on more routine RBC indices such as [Hb]. Thus, the oCOR method may provide better and more appropriate assessments of the factors influencing circulating [Hb]. Therefore, use of the oCOR method is advocated in the future.

This thesis in part questions whether the concept of 'anaemia' needs readdressing, and whether we should be defining volume excess and tHb-mass deficit separately instead? This is based on the findings of Chapter 5, which aimed to determine the degree to which tHb-mass was correlated with [Hb] in different patient populations and across disease states. I have shown that tHb-mass does not correlate with [Hb] in all disease states studied and that the relationship between [Hb] and tHb-mass appears to degrade as patients get sicker with PV a key confounding variable showing wide variability on an individual patient level. Specifically, in disease states most likely to experience expanded plasma volume and shifts in fluid- CLD and CHF, tHb-mass does not correlate with [Hb] in these disease groups. Furthermore, on an individual level, patients may have very similar or the same tHb-mass but vastly different [Hb] and PV. Therefore, measured [Hb], and the diagnosis of anaemia, can be

strongly influenced by (or can largely depend upon) changes in plasma volume. Indeed, tHb-mass can be normal or elevated in anaemia. Furthermore, the inability to readily measure PV and tHb-mass means that patients are generally investigated or treated for a failure of Hb synthesis or of erythrocytosis. This may be inappropriate for a significant number of patients. In an era where personalised medicine is an emerging strategy, these findings may have important clinical implications that warrant further investigation.

In Chapter 6 I explored the relationship between both [Hb] and tHb-mass with measures of objective patient physical fitness quantified by CPET. In a cohort of patients awaiting major elective surgery, I found that tHb-mass was an important determinant of preoperative cardiorespiratory fitness, whereas [Hb] failed to show any significant relationship with physical fitness. Given that tHb-mass is measurable and manipulable, future studies may wish to address whether increasing tHb-mass preoperatively via targeted intervention using intravenous iron supplementation or blood transfusion leads to improved fitness for surgery as quantified by CPET (see future study 1 below). The use of intravenous iron supplementation would also allow the relationship between changes in indices of iron status and tHb-mass to be explored as well as any independent relationship between iron indices and CPET variables to be elucidated. The ongoing UK CAVIAR study, an observational study in patients undergoing cardiac and vascular surgery will address the effects of intravenous iron therapy (received as part of routine care) on changes in [Hb], biomarkers of iron deficiency (e.g ferritin, hepcidin, transferrin saturation) and in a sub-study, changes in total haemoglobin mass (via oCOR method) and functional capacity (assessed by CPET or 6-minute walk test, depending on study site). Additionally, adequately powered and randomised controlled trials are required to see if manipulation of tHb-mass affects surgical outcome, which my thesis was not designed or powered to address but is an interesting area for future research, and appears warranted.

In this thesis PV was derived from the accurate measurement of tHb-mass and not directly quantified using an independent measurement technique. Therefore, future studies (see future study 2 below) may wish to perform an independent assessment of PV using the gold standard method (human serum

albumin labelled with radioactive iodine) to determine the agreement between measured and derived PV values. This may be of importance in studies assessing the effects of treatment not only on red cell indices but also on volume status of patients. For example, the effects of diuretic dose and timing on PV in patients with decompensated HF or ascites related to chronic LD may require a direct measurement of PV if the level of agreement between quantified and derived PV (using the oCOR method) is not of sufficient accuracy. This warrants further investigation.

It is acknowledged that it may not be possible to implement the oCOR method into routine and widespread clinical practice. Instead, it may be an appropriate test in certain clinical contexts where further information on haematological and volume status of patients is required. This may be relevant to patients with anaemia and disease groups who are most likely to suffer expanded plasma volume and shifts in fluid (e.g, heart failure, chronic liver disease, chronic kidney disease), although I have shown that in other disease states such as IBD (not generally considered to commonly experience disturbances in PV) that tHb-mass between individuals can be very similar but [Hb] substantially different. In addition, it would appear justified to use the oCOR method to quantify changes in tHb-mass in cases where the aim of treatment is to optimise Hb and O₂ carrying capacity. In this context, future research may wish to use tHb-mass as the primary haematological outcome variable, for example when longitudinally monitoring the haematological status of patients throughout the perioperative period (see future study 3 below) or during critical illness in patients on the intensive care unit. In addition, how best to normalise tHb-mass and derived blood volume compartments (e.g. body weight, lean body mass, body surface area or using allometric scaling techniques) to allow adequate anthropometric reference values to be established also appears warranted. This is important because if tHb-mass is to be used clinically in the future we need to know what constitutes a 'normal' tHb-mass and therefore what constitutes a tHb-mass deficit or excess. For example, could we established an anaemia classification system based on tHb-mass alongside that for [Hb]? This would require large, adequately powered studies to define such reference values.

Furthermore, in clinical practice if a patient is classified as anaemic based on [Hb] this routinely triggers a series of further investigations (mainly additional blood tests with associated costs to the NHS) to try and elucidate the underlying cause of anaemia and therefore an appropriate treatment to optimise Hb if necessary. However, patients may be anaemic in the context of normal tHb-mass but expanded plasma volume and I have shown that individual patients may have the same tHb-mass but vastly different [Hb] values and may therefore be treated differently if only [Hb] was used as an initial guide. Therefore, could the oCOR method be used to quantify tHb-mass in anaemic patients as an initial screening test? If there is no deficit in tHb-mass, is there a need for subsequent blood tests to be carried out? It would be interesting to find out the number of anaemic patients with electronic blood records at University College Hospital who had additional blood tests (e.g. iron studies, vitamin B12 and folate, thyroid function etc) to find out whether these subsequent results were normal or abnormal? In addition, it would be of interest to elucidate how many patients were treated with a blood transfusion or iron supplementation (oral or intravenous). Thereafter, one could calculate the costs incurred to the NHS from these additional (potentially unnecessary) tests and compare to the cost of performing an oCOR test. In cases where additional blood tests failed to elucidate any abnormal results, would knowledge of a normal tHb-mass have prevented their conduct and/or provided useful additional information? See future study 4 below. This could further be studied prospectively by measuring tHb-mass using the oCOR method in anaemic patients and observing (not intervening) their subsequent standard, routine clinical care and investigations to highlight where tHb-mass would be most useful in this setting.

Future Study 1

Long Title: The effects of intravenous iron on total haemoglobin mass and exercise capacity determined by cardiopulmonary exercise testing in patients undergoing major surgery

Short title: Intravenous iron, tHb-mass and CPET prior to surgery

Phase: Prospective observational study

Population: Patients awaiting major surgery

Number of sites: One

Trial Medication: Non-randomised intravenous iron supplementation (received as routine care)

Primary

objectives:

1. Investigate the effect of intravenous iron supplementation on total haemoglobin mass
2. Investigate the effect of intravenous iron supplementation on anaerobic threshold (AT) and peak oxygen consumption ($\dot{V}O_2$ peak).

Secondary

objectives:

1. Characterise the response of iron indices and hepcidin to intravenous iron treatment

Outcome

measures:

1. Change in total haemoglobin mass (g & g·kg⁻¹)
2. Change in anaerobic threshold (AT) and peak oxygen consumption ($\dot{V}O_2$ peak)
3. Change in markers of iron metabolism and hepcidin

Description of

Study design: This would be a single centre, observational study in patients awaiting major elective surgery. Patients would be tested pre-and post-intravenous iron treatment to determine changes in tHb-mass (measured using the optimised carbon monoxide rebreathing method), markers of iron metabolism and exercise capacity (determined by cardiopulmonary exercise testing (CPET) prior to undergoing operation.

Future Study 2

Long Title: Validity of derived plasma volume (PV) using the optimised carbon monoxide rebreathing method and directly measured PV using the radiolabelled albumin dilution technique.

Short title: Validity of PV using the oCOR and albumin methods

Phase: Observational study

Population: Healthy volunteers

Number of sites: One

Trial Medication: Nil

Primary

objectives: 1. To determine the level of agreement between derived PV using the optimised carbon monoxide rebreathing method and directly measured PV using the gold standard radiolabelled albumin dilution technique

Secondary

objectives: 1. To determine the validity of derived plasma volume using the oCOR method to detect acute PV expansion following intravenous infusion of saline solution compared to the gold standard albumin technique

Outcome

measures: 1. Plasma volume (ml & ml·kg⁻¹)

Description of

Study design: This would be a single centre, observational study in healthy volunteers. Participants would be tested six times in total in this study on two separate days. In part 1, participants would perform the oCOR and albumin methods on the same day to assess the level of agreement between derived and directly measured PV. In part 2, participants would perform both the oCOR and albumin methods pre- and post PV expansion via infusion of saline solution to assess the validity of the oCOR method to detect changes in PV.

Future Study 3

Long Title: Longitudinal assessment of haematological variables during the perioperative period

Short title: Haematological variables during the perioperative period

Phase: Longitudinal observational study

Population: Patients undergoing major surgery

Number of sites: One

Trial Medication: Nil

Primary

objectives:

1. Monitor total haemoglobin mass throughout the perioperative period
2. Monitor blood volume and plasma volume throughout the perioperative period

Secondary

objectives:

1. Monitor routine haematological variables during the perioperative period

Outcome

measures:

1. Change in total haemoglobin mass
2. Change in blood volume and plasma volume
3. Change in haemoglobin concentration and haematocrit

Description of

Study design: This would be a single centre, observational and longitudinal study in patients awaiting major surgery. Patients would have their tHb-mass measured using the oCOR method on the day prior to surgery, the day after surgery and on postoperative days 3, 5, 8, 15, and 21, if the patient remained in hospital until discharge to characterise changes in tHb-mass, blood volume, plasma volume and routine haematological variables (e.g. haemoglobin concentration and haematocrit). This study would allow the underlying causes of postoperative anaemia to be elucidated.

Future Study 4

Long Title:	The prevalence and cost of anaemia in secondary care, a retrospective cohort study
Short title:	Cost of anaemia in the secondary care setting
Phase:	Retrospective observational cohort study
Population:	Anaemic patients with electronic hospital blood records
Number of sites:	Two: University College Hospital and Southampton General Hospital
Trial Medication:	Nil
Primary objectives:	<ol style="list-style-type: none">1. To assess what is currently done in the hospital setting to detect and investigate anaemia2. To assess the economic implications of detecting and subsequently investigating anaemia in the secondary care setting
Secondary objectives:	<ol style="list-style-type: none">1. To determine the cost of measuring tHb-mass using the oCOR method and compare this to the economic costs of detecting and investigating anaemia
Outcome measures:	<ol style="list-style-type: none">1. Economic cost of detecting anaemia using [Hb], MCV, MCHC & RDW.2. Economic cost of investigating anaemia through additional tests (haematinics; total iron binding capacity, ferritin, iron, vitamin B12 and folate, thyroid function tests)3. Comparison of the economic cost of tHb-mass measurement versus cost of detecting, investigating and treating anaemia.
Description of Study design:	This would be a retrospective, multi-centre observational study using electronic patient records in all anaemic patients using anonymised data. Patients would be excluded if they had known bone marrow malignancies. One would assess the prevalence of anaemia in individual

patients (no repeats) based on [Hb], MCV, MCHC, RDW in both the in-patient and outpatient setting with further refinement based on ICD10 diagnosis coding.

Subsequently, one would determine how many patients underwent additional tests (e.g. haematinics; total iron binding capacity, ferritin, iron, vitamin B12 and folate, thyroid function) to elucidate the aetiology of anaemia and in how many patients were these results abnormal? Thus, one could work out the cost per positive finding and conversely the cost per negative finding. In addition, this information could be used to refine current practice. For example, if MCV and MCHC are normal then it is very unlikely that further investigations would be abnormal. Furthermore, if cases where a negative finding was elucidated would confirmation of a normal tHb-mass measurement have prevented their conduct?

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Otto, J.M., Plumb, J.O.M., Wakeham, D., Clissold, E., Loughney, L., Schmidt, W., Montgomery, H.E., Grocott, M.P.W., and Richards, T. Total haemoglobin mass, but not haemoglobin concentration, is associated with preoperative cardiopulmonary exercise testing (CPET) derived oxygen consumption variables. *BJA* (accepted for publication, 27th October 2016).

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Burden, B. J., Pollock, N., Whyte, G. P., Richards, T., Moore, B., Busbridge, M., Srai, S. K., **Otto, J. M.**, and Pedlar, C. R. Impact of Intravenous Iron on Aerobic Capacity and Iron Metabolism in Elite Athletes. *Med Sci Sports Exerc*, 2015. **47**(7):1399-408.

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Abstracts and posters

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preoperative exercise capacity measured by cardiopulmonary exercise testing in patients awaiting major surgery. *Network for the Advancement of Patient Blood Management, Haemostasis and Thrombosis*. 17th Annual Symposium, April 2016.

Pedlar, C. R., Brown, M., **Otto, J. M.**, Drane, A., Finch, J. M., Contursi, M., Shave, R., Wasfy, M., Hutter, A., and Baggish, A. Temporal Sequence of Athlete's Heart Regression During Prescribed Exercise Detraining: Diagnostic Implications. *American College of Cardiology*, December 2016.

Presentations

Otto, J. M., O'Doherty, A. F., Hennis, P. J., Cooper, J. A., Grocott, M. P. W., Snowdon, C., Carlisle, J. B., Swart, M., Richards, T., and Montgomery, H. E. Association between preoperative haemoglobin concentration and cardiopulmonary exercise variables: a multicentre study. Invited presenter at the 6th National Perioperative Cardiopulmonary Exercise Testing (CPET) Forum as part of the Evidence Based-Perioperative Medicine Conference. June 2013.

Otto, J.M. Haemoglobin mass, iron status and cardiorespiratory fitness. Presented to the Robbins Group, Department of Physiology, Anatomy and Genetics at the University of Oxford. November 2014.

Otto, J.M. The importance of haemoglobin mass in the clinical setting. Presented to the Upper Gastrointestinal surgical research meeting at University College Hospital. June 2015.

Otto, J.M. Measurement of total haemoglobin mass in the clinical setting. Presented to the Anaesthetics Department at Papworth Hospital. January 2016.

Otto, J.M., Plumb, J.O.M., Wakeham, D., Montgomery, H.E., Grocott, M.P.W., Richards, T. Total haemoglobin mass is an important determinant of preoperative exercise capacity measured by cardiopulmonary exercise testing in patients awaiting major surgery. *Network for the Advancement of Patient*

Blood Management, Haemostasis and Thrombosis. 17th Annual Symposium, April 2016. Awarded third prize out of 84 accepted abstracts. The 14 best-rated abstracts were invited to present of which I was one.

Otto, J.M. Total haemoglobin mass, but not haemoglobin concentration, is associated with preoperative cardiopulmonary exercise testing (CPET) derived oxygen consumption variables. Presented at the Royal Marsden Anaesthesia and Surgery Education day. November 2016.

Other work

Bruinvels, G., Miles, L., **Otto, J. M.**, Chau, M., and Richards, T. Cochrane Protocol 0224: Iron therapy for iron deficient non-anaemic adults. Started June 2015, work still on going.

Pedlar, C. R., Brown, M., **Otto, J. M.**, Drane, A., Finch, J. M., Contursi, M., Shave, R., Wasfy, M., Hutter, A., and Baggish, A. Temporal Sequence of Athlete's Heart Regression During Prescribed Exercise Detraining: Diagnostic Implications. I was the invited expert on total haemoglobin mass and blood volume for this Boston Marathon Detraining Study. This involved three separate visits to Boston Massachusetts (4-5 days each time) over a 3-month period to monitor the effects of detraining on cardiorespiratory fitness, cardiac structure and function, total haemoglobin mass and blood volume derivatives. I conducted all optimised carbon monoxide rebreathing tests throughout this study.

Appendices

Appendix A – Participant information sheet (Chapter 4)

Information Sheet for Graduate Research Degree (PhD)

You will be given a copy of this information sheet.

Title of Project: Test retest reliability of total haemoglobin mass using the optimised carbon monoxide rebreathing method

This study has been approved by the UCL Research Ethics Committee (Project ID Number): 6654/001

Name Mr James Otto

Work Address Division of Surgery and Interventional Science, c/o Institute of Sport, Exercise and Health, 170 Tottenham Court Road, London, W1T 7HA

Contact Details Email: james.otto@nhs.net, Mobile: 07949 340 801

Introduction

We would like to invite you to take part in a research study. Before you decide whether you would like to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

We wish to assess how reliable (accurate) a new technique is at measuring the total level of haemoglobin in the body, which reflects the capacity of the blood to carry oxygen.

This study is being conducted to fulfil the requirements of a Graduate Research Degree (PhD) being undertaken by Mr James Otto, Division of Surgery and Interventional Science at University College London (UCL).

Haemoglobin concentration [Hb] is a routine and well-established parameter in medicine. However, the measurement of [Hb] can be affected by many factors that may impact its accuracy. A recently developed test measures the total amount of haemoglobin in the body (tHb-mass), which may provide a better, more sensitive marker of oxygen carrying capacity and provide additional information on the clinical status of patients. However, we do not know whether this new technique produces similar results when the same individual is measured on more than one occasion (reliability).

Specifically, the aim of this study is:

1. To assess tHb-mass on two separate occasions to see how similar the values are.

Why have I been invited to take part in this study?

We have chosen you because you are an adult aged 18 years or older and free from chronic medical conditions.

Do I have to take part in the study?

It is up to you to decide whether or not to take part. If you decide to take part you will be given a copy of this information sheet and a signed copy of the consent form to keep and be asked to sign a consent form. If you wish to withdraw from the study you may do so at any time without giving a reason. If you decide not to take part in the study, this will not affect the standard of care you receive. Furthermore, if you chose to withdraw from the study at any point in time your clinical management in the hospital will remain unaffected and identifiable data will be removed from the study database.

What will happen to me if I take part in the study?*Measurement of total haemoglobin mass (tHb-mass)*

tHb-mass will be determined using the optimised carbon monoxide (CO) rebreathing method on two days separated by no more than 7 days. You will be asked to rebreath a small volume of CO for 2 minutes through a mouthpiece. The mouthpiece is attached to specifically designed device for that measures the volume of air entering and leaving the lungs. Before and at two time points after CO exposure, you will have a capillary blood sample taken. In total 3 blood samples are required per test (6 in total).

What data will be collected?

We will obtain a venous blood sample to allow the determination of haemoglobin concentration and haematocrit. To determine your tHb-mass we will collect three capillary blood samples from your earlobe or fingertip to measure carboxyhaemoglobin concentration. The amount of blood required in total is 5-10 ml or 1-2 teaspoons, which is analysed immediately and will not be stored. In the unlikely event that an insufficient amount of blood is obtained by capillary sampling, a venous sample maybe necessary and would require an additional needle stick. Samples will be taken by people experienced in taking blood samples.

What are the benefits of taking part?

It is unlikely that patients will benefit directly from this study.

What are the disadvantages or risks of taking part?

Side effects of carbon monoxide on health and wellbeing are very rare, especially at the very low dose used during our test. However, a very small number of individuals have reported a headache or dizziness. In the unlikely event of these symptoms occurring, a doctor will be consulted at hospital and if necessary will see you. The dose used is less than would occur when in road traffic or after smoking a cigarette. After having blood taken, some very minor bruising sometimes occurs but every effort will be made to prevent this from happening. Samples will be taken by people experienced in taking blood samples.

What will happen if I do not want to carry on with the study?

You are free to withdraw your consent for the study at any time, without giving any reason; this will in no way affect your clinical treatment. If you do decide to withdraw from the study you will be asked to sign an 'early termination' form. All identifiable data will be withdrawn from the study but data that is not identifiable to the research team will be retained and used in this research.

In the (perhaps unlikely) event of a loss of capacity to consent, the research team will retain tissue and personal data collected during this study and continue to use it confidentially for research purposes. This could include further research after the current project has ended.

What if there is a problem?

If you wish to complain, or have any concerns about any aspect of the way you have been approached or treated by members of staff you may have experienced due to your participation in the research, National Health Service or UCL complaints mechanisms are available to you. Please ask the researcher if you would like more information on this.

In the unlikely event that you are harmed by taking part in this study compensation may be available. If you suspect that the harm is the result of the Sponsor's (University College London) or the hospital's negligence then you may be able to claim compensation.

After discussing with your research doctor, please make the claim in writing to the Mr Toby Richards who is the Chief Investigator for the research and is based at UCL Division of Surgery and Interventional Science, 9th Floor, Royal Free Hospital, NW3 2QG. The Chief Investigator will then pass the claim to the Sponsor's Insurers, via the Sponsor's office. You may have to bear the costs of the legal action initially, and you should consult a lawyer about this.

Harm:

We will take every care in the course of this study. We have no reason to believe that you will come to any harm as a result of this research; if you are harmed due to someone's negligence then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms are available to you.

Will my taking part in this study be kept confidential?

At point of entry to the research, each participant will be allocated a unique identification number for the purpose of sample and data storage. Data will be pseudoanonymised; a register linking subject details and identification numbers will be kept to allow subject recall but will remain accessible only to the principal- and co-investigators and held on a password protected computer at University College London or NHS computer. Identifiable data on the register will include name, date of birth and address. Unique identifiers will be used from the time of data collection throughout the whole study duration.

No data will be shared with third parties. All data will remain confidential throughout the whole study and will adhere to Caldicott guidelines.

What if new information becomes available?

Sometimes during the course of a research project, new information becomes available about the subject being studied. Should this happen a member of the research team will tell you about it and discuss with you whether you want to continue in the study.

What will happen to the results of the research study?

Following the completion of data collection from participants the research team will analyse the results. The results will be published in a medical/scientific journal as soon as possible after analysis has finished. You will not be identified in any report or publication. The study results can be sent to you if you wish to receive a report after completion of the study. If so, please contact Mr James Otto via letter at University College London, Division of Surgery and Interventional Science, c/o Institute of Sport, Exercise and Health, 170 Tottenham Court Road, London, W1T 7HA or email james.otto@nhs.net.

Who is organising and funding the research?

Mr James Otto (University College London and University College London Hospital) is organising the study. There is no direct external funding for this research project and therefore we are unable to reimburse any expenses relating to this study.

Ethics Committee review

All research using human subjects is reviewed before an ethics committee before they can proceed. The University College London (UCL) Research Ethics Committee reviewed this proposal.

Contact for further information.

If you have any concerns or questions about the study or the way it has been carried out, you should contact: Mr James Otto, Email: james.otto@nhs.net, Mobile: 07949 340 801

Please discuss the information above with others if you wish or ask us if there is anything that is not clear or if you would like more information.

It is up to you to decide whether to take part or not; choosing not to take part will not disadvantage you in any way. If you do decide to take part you are still free to withdraw at any time and without giving a reason.

All data will be collected and stored in accordance with the Data Protection Act 1998.

Thank you for reading this information sheet and for considering take part in this research.

Appendix B – Informed consent form (Chapter 4)

Informed Consent Form for Graduate Research Degree (PhD)

Please complete this form after you have read the Information Sheet and/or listened to an explanation about the research.

Title of Project: Test retest reliability of total haemoglobin mass using the optimised carbon monoxide rebreathing method

This study has been approved by the UCL Research Ethics Committee (Project ID Number): 6654/001

Thank you for your interest in taking part in this research. Before you agree to take part, the person organising the research must explain the project to you.

If you have any questions arising from the Information Sheet or explanation already given to you, please ask the researcher before you decide whether to join in. You will be given a copy of this Consent Form to keep and refer to at any time.

Participant's Statement

I

- have read the notes written above and the Information Sheet, and understand what the study involves.
- understand that if I decide at any time that I no longer wish to take part in this project, I can notify the researchers involved and withdraw immediately.
- consent to the processing of my personal information for the purposes of this research study.
- understand that such information will be treated as strictly confidential and handled in accordance with the provisions of the Data Protection Act 1998.
- agree that the research project named above has been explained to me to my satisfaction and I agree to take part in this study.

Signed:

Date:

Appendix C – Participant information sheet (Chapter 5)



University College Hospital
Department of Surgery
235 Euston Road
London
NW1 2BU

Direct line: 020 3447 5173
Switchboard: 0845 155 5000/ 020 3456 7890
Ext: 75173

Email: toby.richards@uclh.nhs.uk
Website: www.uclh.nhs.uk

Participant Information Sheet

Version 1.4 – 13th February 2015

Title of Project: Assessment of oxygen carrying capacity in the clinical setting – a pilot
Student's research project

Chief Investigator: **Mr Toby Richards**

Student Researcher: **Mr James Otto**

Introduction

As a patient of University College London Hospitals NHS Trust you are being invited to take part in a research study. Before you decide whether you would like to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

The purpose of this study is to assess the capacity of the blood to carry oxygen around the body. To do this we wish to determine the total level of haemoglobin in the body using a new technique.

This study is being conducted to fulfil the requirements of a Graduate Research Degree (PhD) being undertaken by Mr James Otto, Division of Surgery and Interventional Science at University College London (UCL).

Haemoglobin concentration [Hb] is a routine and well-established parameter in medicine. However, the measurement of [Hb] can be affected by many factors that may impact its accuracy. A recently developed test measures the total amount of haemoglobin in the body (tHb-mass), which may provide a better, more sensitive marker of oxygen carrying capacity and provide additional information on the clinical status of patients. Despite this, there is limited information on total haemoglobin mass in the clinical setting and

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therefore this research aims to investigate the usefulness of total haemoglobin mass in different patient groups.

Specifically, the aims of this study are:

1. To assess [Hb] and tHb-mass in patients to see how well they match each other.
2. To determine whether tHb-mass provides a better assessment of blood oxygen carrying capacity.

Why have I been invited to take part in this study?

We have chosen you because you are a patient at University College London Hospitals NHS Foundation Trust and have one of the following chronic medical conditions; chronic heart failure, liver disease, renal disease or gastrointestinal disease or are due to undergo surgery.

Do I have to take part in the study?

It is up to you to decide whether or not to take part. If you decide to take part you will be given a copy of this information sheet and a signed copy of the consent form to keep and be asked to sign a consent form. If you wish to withdraw from the study you may do so at any time without giving a reason. If you decide not to take part in the study, this will not affect the standard of care you receive. Furthermore, if you chose to withdraw from the study at any point in time your clinical management in the hospital will remain unaffected and identifiable data will be removed from the study database.

What will happen to me if I take part in the study?

Measurement of total haemoglobin mass (tHb-mass)

tHb-mass will be determined using the optimised carbon monoxide (CO) rebreathing method. You will be asked to re breathe a small volume of CO for 2 minutes through a mouthpiece. The mouthpiece is attached to specifically designed device for that measures the volume of air entering and leaving the lungs. Before and at two time points after CO exposure, you will have a blood sample taken. In total 3 blood samples are required.

What data will be collected?

We will collect information from you to help establish your health status, including but not exclusively, your diagnosis, medical history and current medications. Where information is missing, your medical notes will be looked at to obtain this information. To determine your tHb-mass we will collect three blood samples from you to measure carboxyhaemoglobin concentration. The amount of blood required in total is approximately 5-10mls or 1-2 teaspoons, which is analysed immediately and will not be stored. People experienced in taking blood samples will take the samples.

In addition, we wish to collect a urine sample (20-30 ml, 5-6 teaspoons) and venous blood sample (10-15 ml, 3-4 teaspoons), from one needle stick for the purposes of current and future research. These samples will be obtained at the same appointment that your tHb-mass is measured and are additional to routine care. Samples will be labelled with a unique ID number and so will be stored anonymised for personal identifiers. However, members of the research team who have legitimate access to the samples will be

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able to link the ID numbers with corresponding personal information. This is known as a pseudoanonymised system. Samples will be stored in UCL research freezers in the Division of Surgery and Interventional Science in accordance with legislation in the Human Tissue Act (HTA) and UCL policy. We wish to store samples for future research as in recent years, as new technologies and approaches are developed, the analysis of stored samples can contribute substantially to the clinical relevance of research and maximise its value. This research will involve the analysis and use of human DNA in the samples (genetic testing), and proteomic (study of protein structure and function) and metabolomic (study of chemical processes involving metabolites) analyses.

What are the benefits of taking part?

It is unlikely that patients will benefit directly from this study.

What are the disadvantages or risks of taking part?

Side effects of carbon monoxide on health and wellbeing are very rare at the low dose used during our test. However, a very small number of individuals have reported a headache or dizziness. In the unlikely event of these symptoms occurring, a doctor will be consulted at hospital and if necessary will see you. The dose used is less than would occur when in road traffic or after smoking a cigarette. After having blood taken, some very minor bruising sometimes occurs but every effort will be made to prevent this from happening. Samples will be taken by people experienced in taking blood samples.

What will happen if I do not want to carry on with the study?

You are free to withdraw your consent for the study at any time, without giving any reason; this will in no way affect your clinical treatment. If you do decide to withdraw from the study you will be asked to sign an 'early termination' form. All identifiable data will be withdrawn from the study but data that is not identifiable to the research team will be retained and used in this research.

In the (perhaps unlikely) event of a loss of capacity to consent, the research team will retain tissue and personal data collected during this study and continue to use it confidentially for research purposes. This could include further research after the current project has ended.

What if there is a problem?

If you wish to complain, or have any concerns about any aspect of the way you have been approached or treated by members of staff you may have experienced due to your participation in the research, National Health Service or UCL complaints mechanisms are available to you. Please ask your research doctor if you would like more information on this.

In the unlikely event that you are harmed by taking part in this study compensation may be available. If you suspect that the harm is the result of the Sponsor's (University College London) or the hospital's negligence then you may be able to claim compensation.

After discussing with your research doctor, please make the claim in writing to the Mr Toby Richards who is the Chief Investigator for the research and is based at UCL Division of Surgery and Interventional Science, 9th Floor, Royal Free Hospital, NW3 2QG. The Chief Investigator will then pass the claim to the Sponsor's Insurers, via the Sponsor's office. You may have to bear the costs of the legal action initially, and you should consult a lawyer about this.

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Harm:

We will take every care in the course of this study. We have no reason to believe that you will come to any harm as a result of this research; if you are harmed due to someone's negligence then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms are available to you.

Officers from the Patient Advice and Liaison Service (PALS) are available in all hospitals, details at the end of this document. They offer confidential advice, support and information on health-related matters to patients, their families and their carers. You can find your local PALS office on the PALS website <http://www.pals.nhs.uk/officemapsearch.aspx>. Please ask your study doctor if you would like more information on this. Details can also be obtained from the Department of Health website: <http://www.dh.gov.uk>.

Alternatively, the Independent Complaints Advocacy Service (ICAS) is a national service that supports people who wish to make a complaint about their NHS care or treatment. Contact your local ICAS office through PALS, or ask your research nurse for your local contact number. If you wish you can find your local contact number on the following website:-

<http://www.nhs.uk/choiceintheNHS/Rightsandpledges/complaints/Pages/NHScomplaints.aspx>

Will my taking part in this study be kept confidential?

At point of entry to the research, each participant will be allocated a unique identification number for the purpose of sample and data storage. Data will be pseudoanonymised; a register linking subject details and identification numbers will be kept to allow subject recall but will remain accessible only to the principal and co-investigators and held on a password protected computer at University College London or NHS computer. Identifiable data on the register will include name, date of birth and address. Unique identifiers will be used from the time of data collection throughout the whole study duration.

No data will be shared with third parties. All data will remain confidential throughout the whole study and will adhere to Caldicott guidelines.

What if new information becomes available?

Sometimes during the course of a research project, new information becomes available about the subject being studied. Should this happen a member of the research team will tell you about it and discuss with you whether you want to continue in the study.

Will my General Practitioner be notified?

Your GP will be informed of your inclusion in this research study.

What will happen to the results of the research study?

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Following the completion of data collection from participants the research team will analyse the results. The results will be published in a medical/scientific journal as soon as possible after analysis has finished. You will not be identified in any report or publication. The study results can be sent to you if you wish to receive a report after completion of the study. If so, please contact Mr James Otto via letter at University College London, Division of Surgery and Interventional Science, c/o 4th Floor Rockefeller Building, 21 University Street, London, WC1E 6DE or email james.otto@nhs.net.

Who is organising and funding the research?

Mr James Otto (University College London and University College London Hospital) is organising the study. There is no direct external funding for this research project.

Ethics Committee review

All research using human subjects is reviewed before an ethics committee before they can proceed. The London – Camden and Islington Research Ethics Committee reviewed and approved this proposal.

Contact for further information.

If you have any concerns or questions about the study or the way it has been carried out, you should contact: Mr Toby Richards: **020 7679 6454**

If you wish to speak to an independent advisor you should contact:

Patient Advice and Liaison Service (PALS)

Ground Floor Atrium
University College Hospital
235 Euston Road
London
NW1 2BU
T: 0203 447 3042

THANK YOU FOR TAKING TIME TO READ THIS SHEET.

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Appendix D – Informed consent form (Chapter 5)

University College Hospital
Department of Surgery
235 Euston Road
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NW1 2BU

Direct line: 020 3447 5173
Switchboard: 0845 155 5000/ 020 3456 7890
Ext: 75173

Email: tohy.richards@uclh.nhs.uk
Website: www.uclh.nhs.uk

Informed Consent Form

Version 1.4 – 13/02/2015

Title of Project: The assessment of oxygen carrying capacity in the clinical setting – a pilot
Student's research project

Chief Investigator: **Mr Toby Richards**

Student Researcher: **Mr James Otto**

Study number: _____

Please initial box to indicate agreement.

1. I confirm that I have read (or someone has read to me) and understood the Information Sheet dated **13th February 2015 (Version 1.4)** for the above study and have had the opportunity to ask questions and have these answered satisfactorily.

Initial box

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

Initial box

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3. I understand that, relevant sections of my medical notes, and data collected during the study may be looked at by professional responsible individuals from the sponsor of the trial (UCH, UCL, Royal Free Joint Biomedical Research Unit), from regulatory authorities or from the NHS Trust where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. I understand that my personal data will be processed and stored securely in compliance with the 1998 Data Protection Act. In addition, stored data collected during the study may be used for future research and I give permission for this to happen.

Initial box

Informed Consent Form

Version 1.4 – 13/02/2015

Title of Project: The assessment of oxygen carrying capacity in the clinical setting—a pilot

Study number: _____

Please initial box to indicate agreement.

4. Additionally, I understand that the following samples will be collected for research purposes and I consent to these to be collected:

- a. Three blood samples via a cannula/butterfly needle or capillary tube as part of total haemoglobin mass measurement.

Initial box

OR

- b. Seven blood samples via a cannula/butterfly needle or capillary tube as part of validating total haemoglobin mass measurement in patients.

Initial box

- c. One venous blood sample

Initial box

5. I give permission for these samples to be collected and stored, in accordance with the Human Tissue Act, for current and future research.

Initial box

OR

6. I give permission for these samples to be collected and stored for this current research study but I do not give permission for my samples to be stored for use in future research projects.

Initial box

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7. In addition, I consent for DNA testing to be carried out on collected samples for the purposes of current and future research with the appropriate ethical approval.

OR

8. In addition, I consent for DNA testing to be carried out on collected samples for the purpose of this current research project but I do not give permission for my samples to be stored for use in future research projects.

9. I understand that I am gifting my samples to the investigators and in so doing give up all future claims to its use that may include further research with the appropriate ethical approval.

10. I agree to my GP being informed of my participation in the study.

11. I agree to take part in the study requiring three blood samples as part of total haemoglobin mass measurement (see Section 4a above).

OR

12. I agree to take part in the study requiring seven blood samples as part of validating total haemoglobin mass measurement in patients (see Section 4b above).

Name of patient

Date

Signature

Name of person taking consent

Date

Signature

Investigator

Date

Signature

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Appendix E – Participant information sheet (Chapter 6)



University College Hospital
Department of Surgery
235 Euston Road
London
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Direct line: 020 3447 5173
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Ext: 75173

Website: www.uclh.nhs.uk

Participant Information Sheet

Version 1.6 – 10th February 2015

Title of Project: Blood oxygen carrying capacity, iron status, cardiopulmonary exercise testing, and postoperative morbidity in patients awaiting major surgery - a pilot study.

Student's research project

Chief Investigator: **Mr Toby Richards**

Student Researcher: **Mr James Otto**

Introduction

As a patient of University College London Hospitals NHS Trust you are being invited to take part in a research study. Before you decide whether you would like to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

This study is being conducted to fulfil the requirements of a Graduate Research Degree (PhD) being undertaken by **Mr James Otto**, Division of Surgery and Interventional Science at University College London (UCL).

Assessing the physical status of patients before surgery is a difficult task for doctors. A test that helps doctors assess physical capacity is the cardiopulmonary exercise test (CPET). CPET provides an objective assessment of physical status, and more specifically an assessment of heart and lung function; this is useful for identifying risk in surgical patients. Current research suggests that fitter people, measured by CPET, recover more quickly after surgery.

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Haemoglobin, found in red blood cells carries most of the oxygen in the blood and is reduced in patients with anaemia. Haemoglobin is dependent on adequate iron that when reduced can lead to iron deficiency and anaemia. Both anaemia and iron deficiency can result in reduced physical status. Anaemia is routinely defined by measuring haemoglobin concentration [Hb] in the blood and is common in patients awaiting surgery. However, the measurement of [Hb] can be affected by a number of factors that may impact its accuracy. A recently developed test measures the total amount of haemoglobin (tHb-mass) in the body, which may provide more information on the capacity of the blood to carry oxygen than [Hb] alone.

The purpose of this study is to assess the relationship between iron status, tHb-mass and physical capacity using CPET to determine the impact on surgical outcome.

Specifically, the aims of this study are:

1. To assess total haemoglobin mass in surgical patients and establish its effects on exercise capacity and surgical outcome.
2. To investigate the relationship between total haemoglobin mass, iron status and exercise capacity.

Why have I been invited to take part in this study?

We have invited you because you are scheduled to undergo surgery at University College London Hospital and have been referred for a Cardiopulmonary Exercise Test (CPET).

Do I have to take part in the study?

It is up to you to decide whether or not to take part. If you decide to take part you will be given a copy of this information sheet and a signed copy of the consent form to keep and be asked to sign a consent form. If you wish to withdraw from the study you may do so at any time without giving a reason. If you decide not to take part in the study, this will not affect the standard of care you receive. Furthermore, if you chose to withdraw from the study at any point in time your clinical management in the hospital will remain unaffected and your study data will be removed from the study database.

What will happen to me if I take part in the study?

For this study you will undergo one exercise test on a stationary bicycle. This test will be done at the earliest opportunity. Before your exercise test we will ask you standard questions to establish your health status to ensure it is safe to carry out the test. We will also ask you to blow into a tube to assess how your lungs are working. During the test we will analyse the gases in your inhaled and exhaled breath using a facemask. Your heart trace will also be continually monitored and your blood pressure will be monitored regularly. An Exercise Physiologist will be present throughout the test. This appointment will last approximately one hour. This test will be carried out as part of your normal hospital care before surgery.

In addition, you will undergo one test to measure the total amount of haemoglobin in your body. This will entail breathing normally through a tube that is attached to a bag for 2 minutes. The bag contains a very small amount of carbon monoxide (CO) that is rebreathed during this short period. Before and at defined time points after CO exposure, we will take blood samples to measure carboxyhaemoglobin concentration (COHb [%]). In total 3 blood samples are required. This appointment will last

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approximately 30 minutes and where possible, we will try to arrange this appointment to fit in with other hospital appointments that you have but this cannot be guaranteed.

Finally, a blood test will be carried out to determine your iron status. This will be carried out at the same time as your appointment to measure tHb-mass and will not involve an extra needle stick.

After surgery, your recovery will be monitored until you are discharged from hospital.

What data will be collected?

During CPET we will make observations about how well you are using the oxygen when you are breathing. We will collect information from you to help establish your health status, including but not exclusively, your diagnosis, medical history and current medications. Where information is missing, your medical notes will be looked at to obtain this information. To determine your tHb-mass we will collect three capillary blood samples from your earlobe to measure carboxyhaemoglobin concentration. The amount of blood required in total is 5-10 ml or 1-2 teaspoons, which is analysed immediately and will not be stored. In the unlikely event that an insufficient amount of blood is obtained by capillary sampling, a venous sample may be necessary and would require an additional needle stick. Samples will be taken by people experienced in taking blood samples.

After you have had your surgery we will record information about your hospital stay, including details of certain treatments you received, where you were cared for whilst in hospital and for how long. We will monitor your progress by looking at your electronic records at several time points after your surgery, up to your discharge from hospital.

In addition, we wish to collect a urine sample (20-30 ml, 5-6 teaspoons) and venous blood sample (10-15 ml, 3-4 teaspoons), from one needle stick for the purposes of current and future research. These samples will be obtained at the same appointment that your tHb-mass is measured and are additional to routine care. Samples will be labelled with a unique ID number and so will be stored anonymised for personal identifiers. However, members of the research team who have legitimate access to the samples will be able to link the ID numbers with corresponding personal information. This is known as a pseudoanonymised system. Samples will be stored in UCL research freezers in the Division of Surgery and Interventional Science in accordance with legislation in the Human Tissue Act (HTA) and UCL policy. We wish to store samples for future research as in recent years, as new technologies and approaches are developed, the analysis of stored samples can contribute substantially to the clinical relevance of research and maximise its value. This research will involve the analysis and use of human DNA in the samples (genetic testing), and proteomic (study of protein structure and function) and metabolomic (study of chemical processes involving metabolites) analyses.

What are the benefits of taking part?

It is unlikely that you will benefit by taking part in this research. However, the addition of measuring iron status may highlight those who are iron deficient who might otherwise not have been tested for this condition. In this case, your direct care team may implement appropriate treatment if necessary. In addition, the results of this study may help future patients who have major surgery.

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What are the disadvantages or risks of taking part?

The cardiopulmonary exercise test is part of the routine management of patients undergoing this type of surgery at this hospital and many hospitals across the UK. There is a small risk (1:10,000) associated with CPET (for example risk of an abnormal heart rhythm brought on by exercise or heart attack). Highly experienced physiologists who are trained in advanced life support carefully monitor exercise tests. If you have any significant changes to your heart rhythm during the test, we will refer you to a Cardiologist for an independent review.

There exists the possibility that your muscles may feel achy or sore following exercise but any soreness or aches should subside within a day or two. Your safety will be continually monitored during your visits to the CPET laboratory by the researchers.

Side effects of carbon monoxide on health and wellbeing are very rare, especially at the low dose used during our test. However, a very small number of individuals have reported a headache or dizziness. The dose used is less than would occur when in road traffic or after smoking a cigarette.

What will happen if I do not want to carry on with the study?

You are free to withdraw your consent for the study at any time, without giving any reason; this will in no way affect your clinical treatment. If you do decide to withdraw from the study you will be asked to sign an 'early termination' form. All identifiable data will be withdrawn from the study but data that is not identifiable to the research team will be retained and used in this research study.

In the (perhaps unlikely) event of a loss of capacity to consent, the research team will retain tissue and personal data collected during this study and continue to use it confidentially for research purposes. This could include further research after the current project has ended.

What if there is a problem?

If you wish to complain, or have any concerns about any aspect of the way you have been approached or treated by members of staff you may have experienced due to your participation in the research, National Health Service or UCL complaints mechanisms are available to you. Please ask your research doctor if you would like more information on this.

In the unlikely event that you are harmed by taking part in this study compensation may be available. If you suspect that the harm is the result of the Sponsor's (University College London) or the hospital's negligence then you may be able to claim compensation.

After discussing with your research doctor, please make the claim in writing to the Mr Toby Richards who is the Chief Investigator for the research and is based at UCL Division of Surgery and Interventional Science, 9th Floor, Royal Free Hospital, NW3 2QG. The Chief Investigator will then pass the claim to the Sponsor's Insurers, via the Sponsor's office. You may have to bear the costs of the legal action initially, and you should consult a lawyer about this.

Harm:

We will take every care in the course of this study. We have no reason to believe that you will come to any harm as a result of this research; however, if you are harmed by taking part in this research project, there are no special compensation arrangements available to you. If you are harmed due to someone's

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negligence then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms are available to you.

Officers from the Patient Advice and Liaison Service (PALS) are available in all hospitals, details at the end of this document. They offer confidential advice, support and information on health-related matters to patients, their families and their carers. You can find your local PALS office on the PALS website <http://www.pals.nhs.uk/officemapsearch.aspx>. Please ask your study doctor if you would like more information on this. Details can also be obtained from the Department of Health website: <http://www.dh.gov.uk>.

Alternatively, the Independent Complaints Advocacy Service (ICAS) is a national service that supports people who wish to make a complaint about their NHS care or treatment. Contact your local ICAS office through PALS, or ask your research nurse for your local contact number. If you wish you can find your local contact number on the following website:-

<http://www.nhs.uk/choiceintheNHS/Rightsandpledges/complaints/Pages/NHScomplaints.aspx>

Will my taking part in this study be kept confidential?

All information, which is collected, about you during the course of the research will be kept strictly confidential at all times. The information will be held securely on paper at the University College Hospital, London. Data will be entered onto the study database on a password-protected computer, under the provisions of the 1998 Data Protection Act. A unique identification number will replace identifiable patient information. This will be used on the data collection forms; therefore any information about you that leaves the hospital will not be recognisable. We are required to keep the data for a minimum of 5 years after the study has been completed. It will be stored securely during this time in a locked filing cabinet in a locked office and electronically on a password protected, encrypted computer in a locked office.

What if new information becomes available?

Sometimes during the course of a research project, new information becomes available about the subject being studied. Should this happen a member of the research team will tell you about it and discuss with you whether you want to continue in the study.

Will my General Practitioner be notified?

Your GP will be informed of your inclusion in this research study.

What will happen to the results of the research study?

Following the completion of data collection from participants the research team will analyse the results. The results will be published in a medical/scientific journal as soon as possible after analysis has finished. You will not be identified in any report or publication. The study results can be sent to you if you wish to receive a report after completion of the study. If so, please contact Mr James Otto via letter at University College London, Division of Surgery and Interventional Science, c/o 4th Floor Rockefeller Building, 21 University Street, London, WC1E 6DE or email james.otto@nhs.net.

Who is organising and funding the research?

Sir Robert Naylor, chief executive

Mr James Otto (University College London and University College London Hospital) is organising the study. There is no direct external funding for this research project.

Ethics Committee review

All research using human subjects is reviewed before an ethics committee before they can proceed. The London – Camden and Islington Research Ethics Committee reviewed and approved this proposal.

Contact for further information.

If you have any concerns or questions about the study or the way it has been carried out, you should contact: Mr James Otto

Email: james.otto@nhs.net

If you wish to speak to an independent advisor you should contact:

Patient Advice and Liaison Service (PALS)

Ground Floor Atrium
University College Hospital
235 Euston Road
London
NW1 2BU
T: 0203 447 3042

THANK YOU FOR TAKING TIME TO READ THIS SHEET.

Sir Robert Naylor, chief executive

Appendix F – Informed consent form (Chapter 6)

University College Hospital
Department of Surgery
235 Euston Road
London
NW1 2BU

Direct line: 020 3447 5173
Switchboard: 0845 155 5000/ 020 3456 7890
Ext: 75173

Email: tooby.richards@uclh.nhs.uk
Website: www.uclh.nhs.uk

Informed Consent Form

Version 1.6 – 10th February 2015

Title of Project: Blood oxygen carrying capacity, iron status and cardiopulmonary exercise testing in patients awaiting major surgery, and their relationship to postoperative morbidity: a pilot study

Student's research project

Chief Investigator: **Mr Toby Richards**

Student Researcher: **Mr James Otto**

Study number: _____

Please initial box to indicate agreement.

1. I confirm that I have read (or someone has read to me) and understood the Information Sheet dated **10th February 2015 (Version 1.6)** for the above study and have had the opportunity to ask questions and have these answered satisfactorily.

Initial box

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

Initial box

3. I understand that, relevant sections of my medical notes, and data collected during the study may be looked at by professional responsible individuals from the sponsor of the trial (UCH,

Sir Robert Naylor, chief executive



University
College
Hospital

National Hospital
for Neurology and
Neurosurgery

Eastman
Dental
Hospital

Royal National
Throat, Nose
and Ear Hospital

Heart
Hospital

Royal London
Hospital for
Integrated Medicine

UCL, Royal Free Joint Biomedical Research Unit), from regulatory authorities or from the NHS Trust where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. I understand that my personal data will be processed and stored securely in compliance with the 1998 Data Protection Act.

Initial box

Informed Consent Form

Version 1.6 – 10th February 2015

Title of Project: Blood oxygen carrying capacity, iron status and cardiopulmonary exercise testing in patients awaiting major surgery, and their relationship to postoperative morbidity: a pilot study

Study number: _____

Please initial box to indicate agreement.

4. Additionally, I understand that the following samples will be collected for research purposes and I consent to these to be collected:

- a. Three blood samples via a cannula/butterfly needle or capillary tube as part of total haemoglobin mass measurement.

Initial box

- b. One venous blood sample

Initial box

5. I give permission for these samples to be collected and stored, in accordance with the Human Tissue Act, for current and future research.

Initial box

OR

6. I give permission for these samples to be collected and stored for this current research study but I do not give permission for my samples to be stored for use in future research projects.

Initial box

7. In addition, I consent for DNA testing to be carried out on collected samples for the purposes of current and future research with the appropriate ethical approval.

Initial box

OR

Sir Robert Naylor, chief executive

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University College Hospital Consent form, Version 1.6, 10th February 2015

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8. In addition, I consent for DNA testing to be carried out on collected samples for the purpose of this current research project but I do not give permission for my samples to be stored for use in future research projects.

Initial box

9. I understand that I am gifting my samples to the investigators and in so doing give up all future claims to its use that may include further research with the appropriate ethical approval.

Initial box

10. I agree to my GP being informed of my participation in the study.

Initial box

11. I agree to take part in the above study.

Initial box

Name of patient

Date

Signature

Name of person taking consent

Date

Signature

Investigator

Date

Signature

Sir Robert Naylor, chief executive

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University College Hospital Consent form, Version 1.6, 10th February 2015

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Appendix G – Association between preoperative haemoglobin concentration and cardiopulmonary exercise variables: a multicentre study

Otto et al. *Perioperative Medicine* 2013, **2**:18
<http://www.perioperativemedicinejournal.com/content/2/1/18>



RESEARCH

Open Access

Association between preoperative haemoglobin concentration and cardiopulmonary exercise variables: a multicentre study

James M Otto^{1,2*}, Alasdair F O'Doherty², Philip J Henniss², Jackie A Cooper³, Michael PW Grocott^{2,4}, Chris Snowdon⁶, John B Carlisle⁷, Michael Swart⁷, Toby Richards¹ and Hugh E Montgomery⁵

Abstract

Background: Preoperative anaemia and low exertional oxygen uptake are both associated with greater postoperative morbidity and mortality. This study reports the association among haemoglobin concentration ([Hb]), peak oxygen uptake ($\dot{V}O_2$ peak) and anaerobic threshold (AT) in elective surgical patients.

Methods: Between 1999 and 2011, preoperative [Hb] and cardiopulmonary exercise tests were recorded in 1,777 preoperative patients in four hospitals. The associations between [Hb], $\dot{V}O_2$ peak and AT were analysed by linear regression and covariance.

Results: In 436 (24.5%) patients, [Hb] was $<12 \text{ g dl}^{-1}$ and, in 83 of these, $<10 \text{ g dl}^{-1}$. Both AT and $\dot{V}O_2$ peak rose modestly with increasing [Hb] ($r^2 = 0.24$, $P < 0.0001$ and $r^2 = 0.30$, $P < 0.0001$, respectively). After covariate adjustment, an increase in [Hb] of one standard deviation was associated with a 6.7 to 9.7% increase in $\dot{V}O_2$ peak, and a rise of 4.4 to 6.0% in AT. Haemoglobin concentration accounted for 9% and 6% of the variation in $\dot{V}O_2$ peak and AT respectively.

Conclusions: To a modest extent, lower haemoglobin concentrations are independently associated with lower oxygen uptake during preoperative cardiopulmonary exercise testing. It is unknown whether this association is causative.

Keywords: Anaemia, Cardiopulmonary exercise testing, CPET, Haemoglobin concentration, Oxygen uptake, Surgery

Background

Increased mitochondrial oxygen uptake requires increased cellular oxygen delivery. When oxygen delivery, or utilization, fails to meet metabolic demand, anaerobic cytoplasmic metabolism significantly augments aerobic mitochondrial ATP generation with a consequent increase in lactic acid production and accumulation. It has been suggested that an imbalance in oxygen demand-supply contributes to the point during cardiopulmonary exercise testing (CPET) known as the anaerobic threshold (AT) [1], although this physiological underpinning is not without controversy [2,3]. Major surgery places

substantial metabolic demands upon the patient and may increase resting oxygen uptake from an average preoperative value of $110 \text{ ml min}^{-1} \text{ m}^{-2}$ to approximately $170 \text{ ml min}^{-1} \text{ m}^{-2}$ [4,5]. Lower preoperative exertional oxygen uptake ($\dot{V}O_2$), both the peak and that noted at AT, are associated with postoperative morbidity and mortality [6-8].

Each gram of haemoglobin carries 1.34 ml of oxygen when fully saturated. Anaemia, commonly defined as a haemoglobin concentration ([Hb]) below 13 g dl^{-1} (males) and 12 g dl^{-1} (non-pregnant females), reduces the blood's oxygen carriage capacity. Anaemia is common amongst preoperative patients, with a prevalence ranging from 5% to 76% [9]. Anaemia and blood transfusion are associated with poor postoperative outcomes [10-16].

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Oxygen delivery limits maximal oxygen uptake during exercise under normoxic conditions [17,18]. Increases in [Hb] increase $\dot{V}O_2$ peak, whilst acute reductions in [Hb] lower $\dot{V}O_2$ peak and endurance performance [19-21]. However, the extent to which postoperative outcomes are dependent upon interactions between [Hb] and $\dot{V}O_2$ is unknown. We analysed a large multicentre dataset to explore the relationship between preoperative [Hb] and $\dot{V}O_2$.

Methods

Patient population

We analysed cardiopulmonary exercise (CPET) data collected between December 1999 and February 2011 by four centres: University College London Hospitals NHS Trust (UCLH); the Whittington Hospital NHS Trust; Torbay Hospital, South Devon Healthcare NHS Foundation Trust; and the Freeman Hospital, Newcastle Upon Tyne Hospitals NHS Foundation Trust. Patients had been routinely tested as part of the clinical service before elective surgery: maxillofacial, hepatobiliary, vascular, upper gastrointestinal, colorectal, orthopaedic, bariatric and other specialties (mainly urological).

Following discussion among the researchers and the local Research and Development and clinical governance departments, formal ethical approval was waived and confirmation of audit status granted due to the nature of the data collection.

Cardiopulmonary exercise testing

Across all testing sites, CPET was performed according to the American Thoracic Society/American College of Chest Physicians (ATS/ACCP) guidelines, under stable environmental conditions, with continuous 12 lead ECG monitoring and in the presence of a clinician [22].

Patients pedalled an electromagnetically-braked cycle ergometer (Lode BV, Groningen, Netherlands), with breath-by-breath respiratory gas analysis performed by various machines, (UCL-Cortex Biophysik, Leipzig, Germany; Torbay and Newcastle-Medical Graphics, Minnesota, USA) calibrated according to ATS/ACCP guidelines. During exercise, oxygen uptake ($\dot{V}O_2$) and carbon dioxide output ($\dot{V}CO_2$) were recorded, together with respiratory rate, tidal volume, ventilation and end-tidal gas tensions.

A 3-minute rest period followed fitting of relevant equipment, after which unloaded cycling was performed at a cadence of 60 to 70 rpm for 3 minutes. Thereafter, patients performed a symptom-limited continuous incremental exercise ramp protocol, determined by the physiologist or clinician on the basis of predictive work rate algorithms and patient-reported activity levels [23]. The test continued (usually for 8 to 12 minutes) until volitional exhaustion occurred, or the patient was unable to

maintain a cadence of 40 rpm for more than 30 seconds despite encouragement. The clinician stopped the test if the patient developed a sign or symptom listed in the ATS/ACCP guidelines, which included: new arrhythmia; more than 2 mm of ST elevation or depression on the ECG; an arterial blood pressure of more than 250 mm Hg systolic or 120 mm Hg diastolic (see 2003 ATS/ACCP statement on Cardiopulmonary Exercise Testing for an exhaustive list) [23]. Following termination of CPET, patients were encouraged to perform a 'warm-down' period of unloaded cycling.

The anaerobic threshold was estimated by an exercise physiologist or consultant physician, both experienced in CPET interpretation, using a combination of the modified V-slope, ventilatory equivalents and end-tidal pressure methods [24], which improves the rigor of AT detection. The $\dot{V}O_2$ peak was recorded as the highest average $\dot{V}O_2$ over the final 30 s period [25]. The ventilatory equivalents for carbon dioxide ($\dot{V}E/\dot{V}CO_2$) and oxygen ($\dot{V}E/\dot{V}O_2$) were recorded at the AT [26].

We recorded age, sex, height, weight, body mass index (BMI, kg m^{-2}) and Lee's Revised Cardiac Risk Index (RCRI) from patients' medical histories [27]. In addition, serum creatinine, obtained from hospital electronic records systems, was used as an index of renal function. At UCLH and the Whittington Hospital, [Hb] was measured on the day of CPET (HemoCue AB, Angelholm, Sweden). Preoperative [Hb] was recorded from hospital electronic record systems within 3 days of CPET at Newcastle and at the time of pre-assessment (usually within 4 weeks of CPET) at Torbay. No patient received a blood transfusion between the [Hb] measurement and the CPET.

Statistical analysis

Statistical analysis was performed using Stata Version 11 (StataCorp, Texas, USA). Gaussian distributions of the data were verified by the Kolmogorov-Smirnov test, in conjunction with visual inspection of histogram charts. A difference between data was considered significant if $P < 0.05$. We transformed seven continuous variables with skewed distributions by taking their \log_{10} : weight, BMI, AT, $\dot{V}O_2$ peak, $\dot{V}E/\dot{V}CO_2$, $\dot{V}E/\dot{V}O_2$, and creatinine. These variables are presented as geometric means and approximate SD.

Historically, measurements of oxygen uptake have been indexed to body mass ($\text{ml kg}^{-1} \text{min}^{-1}$), as it allows comparisons between individuals [28,29]. However, this value may still vary with body mass [30-32]. We therefore adjusted the measured oxygen uptake by raising the body mass to a power determined by allometric scaling using the power function ratio (Y/X^b) [29,33]. Specifically, the allometric relationship between body

size and performance measure (AT or $\dot{V}O_2$ peak) is determined by the allometric equation below (see equation 1), where Y is AT or $\dot{V}O_2$ peak, X is body mass, β is a scaling exponent, a is the proportionality constant (intercept), and ϵ is the multiplicative error term, which overcomes the problem of heteroscedasticity [34].

$$Y = aX^\beta \epsilon \quad (1)$$

The allometric relationship between body mass (X) and fitness parameter (Y) is expressed using the logarithmic transformation of equation 1 so that

$$\log Y = \beta \cdot \log \text{mass} + \log a + \log \epsilon \quad (2)$$

where β is the sample specific slope of the linear least squares regression line calculated by log-linear regression analysis (that is, scaling exponent β was 0.83 in the current study) and $\log a$ is the equivalent constant value (a) [34]. We further built models by adjustment for the determinant variable (AT and $\dot{V}O_2$ peak) to potential confounders. Three levels of increasing adjustment were used: i) a basic adjustment for testing site; ii) an extended adjustment for testing site, age and sex; and iii) a fully adjusted model for all known confounders (testing site, age, sex, revised cardiac risk index, diabetes, creatinine and operation category). Results were standardised for testing centre by the inclusion of dummy variables in the regression model.

The effect size was expressed as the percentage increase in $\dot{V}O_2$ for a 1 g dl⁻¹ (or one standard deviation) increase in [Hb]. Partial correlations between [Hb] and CPET markers were performed controlling for confounding variables. Regression models assessed the associations between $\dot{V}O_2$ and [Hb] and the proportion of variance in oxygen uptake explained by variation in [Hb]. Covariance models were analysed with [Hb] as a clinically relevant categorical variable ([Hb] <10 g dl⁻¹; 10 to 12 g dl⁻¹; >12 g dl⁻¹), as similarly described [15,35]. The adjusted values for $\dot{V}O_2$ generated by the model were transformed back to the original scale to give geometric means and approximate standard deviations by [Hb] group.

Results

We analysed data from 1,777 patients (1,108 male) undergoing various operations: 549 vascular (31%), 530 colorectal (30%), 337 bariatric (19%), 75 upper gastrointestinal (4%), 66 hepatobiliary (4%), 48 maxillofacial (3%) and 172 other operations (9%). Contributions from each centre were as follows: 804 UCLH; 484 Whittington; 305 Torbay; 184 Newcastle. The mean (SD) $\dot{V}O_2$ peak and AT were 15.5 (5.9) and 11.2 (3.5) ml kg⁻¹ min⁻¹ respectively. The $\dot{V}E/\dot{V}O_2$ and $\dot{V}E/\dot{V}CO_2$ at AT were 25.9 (6.4) and 30.8 (6.4). The AT was not identified in 146 patients (8.2%).

Mean (SD) [Hb] and creatinine were 13.2 (1.8) g dl⁻¹ and 82 (30) $\mu\text{mol l}^{-1}$. Table 1 lists other physical characteristics.

Relationships between haemoglobin concentration and oxygen uptake

Figure 1 graphs the increase in unadjusted $\dot{V}O_2$ peak with [Hb], whilst Figure 2 shows the relationship between unadjusted oxygen uptake at AT and [Hb]. The $\dot{V}O_2$ peak and AT increased across each [Hb] group (Table 2). More patients awaiting colorectal surgery were anaemic: in 144/530 (27%) of these, the [Hb] was 10 to 12 g dl⁻¹ and in 32/530 (6%) the [Hb] was <10 g dl⁻¹.

Haemoglobin concentration showed weak correlation with $\dot{V}O_2$ peak (r^2 0.30, P <0.0001) and AT (r^2 0.24, P <0.0001), after adjusting for weight and testing centre. Correlations between [Hb] and $\dot{V}O_2$ peak at each site were: Whittington (r^2 0.30, P <0.0001), Torbay (r^2 0.16, P = 0.004), UCLH (r^2 0.31, P <0.0001), and Newcastle (r^2 0.33, P <0.0001). Correlations between [Hb] and AT at each site were: Whittington (r^2 0.23, P <0.0001), Torbay (r^2 0.23, P <0.0001), UCLH (r^2 0.24, P <0.0001), and Newcastle (r^2 0.33, P <0.0001).

Regression models

An increase in [Hb] by one SD was associated with a 9.7% (95% CI, 8.2 to 11.3) increase in $\dot{V}O_2$ peak after adjusting for weight (P <0.0001), which was reduced to 6.7% (95% CI, 5.4 to 7.8) after adjusting for age, sex, weight and testing centre (P <0.0001). The percentage of the variance in $\dot{V}O_2$ peak explained by [Hb] was 8.9% (P <0.0001) after adjusting for weight, and 5.5% (P <0.0001) after adjusting for age, sex, weight and testing site. An increase in [Hb] by one SD was associated with a 6.0% (95% CI, 4.8 to 7.3) increase in AT after adjusting for weight (P <0.0001), which was reduced to 4.4% (95% CI, 3.3 to 5.5) after adjusting for age, sex, weight and testing centre (P <0.0001). The percentage of variance in AT explained by [Hb] was 5.9% (P 0.0001) after weight adjustment, reducing to 3.5% (P <0.0001) after adjusting for age, sex, weight and testing centre.

Table 1 Physical characteristics of the whole-study cohort

	N	Mean	SD
Age (yr)	1,777	61.9	15.8
Height (cm)	1,776	169.0	9.1
Weight (kg)	1,775	83.7	23.6
BMI (kg m ⁻²)	1,774	29.4	8.3
$\dot{V}O_2$ peak (ml min ⁻¹)	1,774	1300	500
AT (ml min ⁻¹)	1,631	940	300

BMI body mass index, AT anaerobic threshold, $\dot{V}O_2$ peak, peak oxygen uptake.

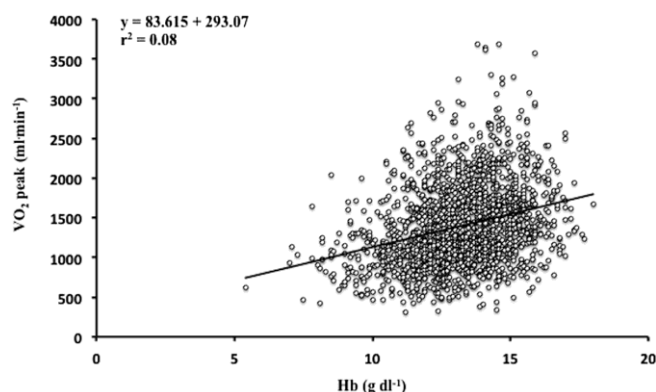


Figure 1 Linear regression between unadjusted $\dot{V}O_2$ peak (ml min^{-1}) and haemoglobin concentration ([Hb]), $n = 1,774$.

Analysis of covariance across haemoglobin group

$\dot{V}O_2$ peak and AT increased across [Hb] groups, after adjusting for confounding variables ($P < 0.0001$, Table 3 and Figure 3).

We analysed allometrically scaled $\dot{V}O_2$ peak across individual surgical cohorts, with and without adjustment for confounders. $\dot{V}O_2$ peak did not differ in hepatobiliary patients across [Hb] groups. $\dot{V}O_2$ peak increased across each [Hb] group for colorectal, bariatric and other (mainly urological) patients, after adjusting for all confounders (testing centre, age, sex, weight, RCRI, diabetes, serum creatinine and operation category). $\dot{V}O_2$ peak increased across each [Hb] classification for upper gastrointestinal and vascular patients after adjustment for testing centre, age, sex and weight, but not when additional confounders

(RCRI, diabetes, serum creatinine and operation category) were added. $\dot{V}O_2$ peak increased with each [Hb] group for maxillofacial patients after adjusting for testing and weight, but not when additional confounders were added to the model.

Discussion

To our knowledge, this is the largest study to explore the relationship between oxygen uptake and haemoglobin concentration in the clinical setting, and the first to control for body mass with allometric (log-linear) scaling. Oxygen uptake at peak exercise and at the anaerobic threshold (AT) increased with haemoglobin concentration [Hb], after adjusting for measured confounding variables.

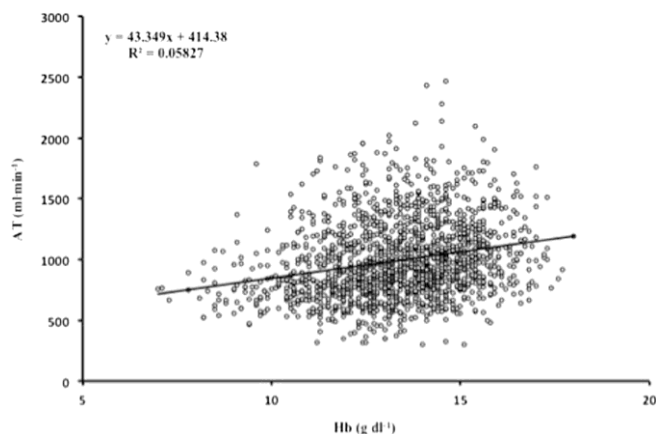


Figure 2 Linear regression between unadjusted oxygen uptake at anaerobic threshold (AT) (ml min^{-1}) and haemoglobin concentration ([Hb]), $n = 1,631$.

Table 2 Physical characteristics across haemoglobin concentration ([Hb]) group (<10 g dl⁻¹; Hb 10 to 12 g dl⁻¹; Hb >12 g dl⁻¹)

	[Hb]		
	<10 g dl ⁻¹	10 to 12 g dl ⁻¹	>12 g dl ⁻¹
N	83	353	1341
Age (yr)	63.5 (16.8)	61.8 (16.4)	61.8 (15.5)
Height (cm)	165.8 (9.2)	166.1 (9.2)	170.0 (8.9)
Weight (kg)	73.3 (22.1)	78.9 (23.7)	85.7 (23.3)
BMI (kg m ⁻²)	26.8 (8.1)	28.7 (8.7)	29.7 (8.2)
Creatinine (μmol l ⁻¹)	79.8 (39.0)	78.7 (29.5)	83.4 (28.9)
$\dot{V}O_2$ peak (ml min ⁻¹) ^a	960 (330)	1150 (430)	1380 (500)
$\dot{V}O_2$ peak (ml kg ⁻¹ min ⁻¹) ^b	13.0 (4.5)	14.5 (5.4)	16.1 (5.8)
$\dot{V}O_2$ peak (ml kg ^{-0.83} min ⁻¹) ^{c, g}	27.8 (6.9)	31.5 (8.1)	34.3 (8.1)
AT (ml min ⁻¹) ^d	760 (190)	860 (260)	970 (310)
AT (ml kg ⁻¹ min ⁻¹) ^e	10.3 (2.5)	10.9 (3.2)	11.3 (3.6)
AT (ml kg ^{-0.83} min ⁻¹) ^{f, g}	21.3 (4.3)	23.5 (4.8)	24.4 (4.8)
$\dot{V}E/\dot{V}O_2$	26.7 (4.8)	25.3 (5.4)	26.0 (6.8)
$\dot{V}E/\dot{V}CO_2$	31.7 (5.9)	30.2 (5.9)	31.0 (6.6)

BMI body mass index, $\dot{V}E/\dot{V}O_2$ and $\dot{V}E/\dot{V}CO_2$, ventilatory equivalents for oxygen and carbon dioxide at the AT; AT, anaerobic threshold; $\dot{V}O_2$ peak, peak oxygen uptake. Values are mean (SD). ^aANOVA, $P < 0.0001$; linear trend, $P < 0.0001$. ^bANOVA, $P < 0.0001$; linear trend, $P < 0.0001$. ^cANOVA, $P < 0.0001$; linear trend, $P < 0.0001$; ^dANOVA, $P < 0.0001$; ^elinear trend, $P < 0.0001$; ^flinear trend, $P < 0.0001$; ^gAdjusted for weight, testing site, age, sex, revised cardiac risk index, diabetes, creatinine and operation category.

The [Hb] was 10 to 12 g dl⁻¹ in 353 patients (20%) and <10 g dl⁻¹ in 83 (5%) patients. Other studies have reported rates of anaemia between 5% and 76%, a range partly dependent upon the indication for surgery and definition of anaemia [9]. The American College of Surgeons' National Surgical Quality Improvement Program (ACS NSQIP) recently reported a similar prevalence of preoperative anaemia (30.4%) [15].

The mean AT of 11 ml kg⁻¹ min⁻¹ is similar to that reported by other studies of preoperative populations [7,36,37]. However, this value is 2 to 3 ml kg⁻¹ min⁻¹ less

than gender-specific reference values for age and height [38]. The mean $\dot{V}O_2$ peak in our population, 15.5 ml kg⁻¹ min⁻¹, is 11 ml kg⁻¹ min⁻¹ less than gender-specific reference values for age and height [38,39]. The aim of allometric scaling is to appropriately account for body size (that is, the scaled variable no longer varies with body size) [40]. Oxygen uptake is usually reported per unit body mass, ml kg⁻¹ min⁻¹, a scale that requires further adjustment for body size [41]. In the obese, oxygen uptake expressed as ml kg⁻¹ min⁻¹ underestimates fitness and overestimates risk [42]. In the cachectic patient this scale overestimates fitness and underestimates risk [43].

Both AT and $\dot{V}O_2$ peak increased with [Hb], for the whole population, across individual testing sites and across all groups, except hepatobiliary surgery. However, this relationship was weak and although being highly statistically significant does not necessarily reflect a magnitude of clinical association. Nonetheless, an increase in [Hb] by one standard deviation (that is, 1.8 g dl⁻¹ rise in [Hb]) was associated with a 9.7% and 6.0% increase in weight-adjusted $\dot{V}O_2$ peak and AT. The [Hb] explained 9% and 6% of the variance in $\dot{V}O_2$ peak and AT respectively. The increase in AT and $\dot{V}O_2$ peak with [Hb] may be due to increased oxygen-carrying capacity, or patients who are not anaemic exercising more than patients who are anaemic, or due to confounding. For instance, sick patients may be both anaemic and less fit. In addition, differences in AT, to some extent (although probably small), may be explained by inherent variations in measurement and/or interpretation or physiological context [44].

The cause of anaemia may be important. The most common cause is reported to be chronic disease, the severity of which being related to the degree of systemic inflammation [45,46]. Features of anaemia due to chronic disease include reduced red cell survival, impaired erythropoiesis, and impaired iron metabolism, all of which may reduce AT and $\dot{V}O_2$ peak, directly or in combination [47]. Iron status was not routinely assessed

Table 3 Allometrically scaled oxygen uptake

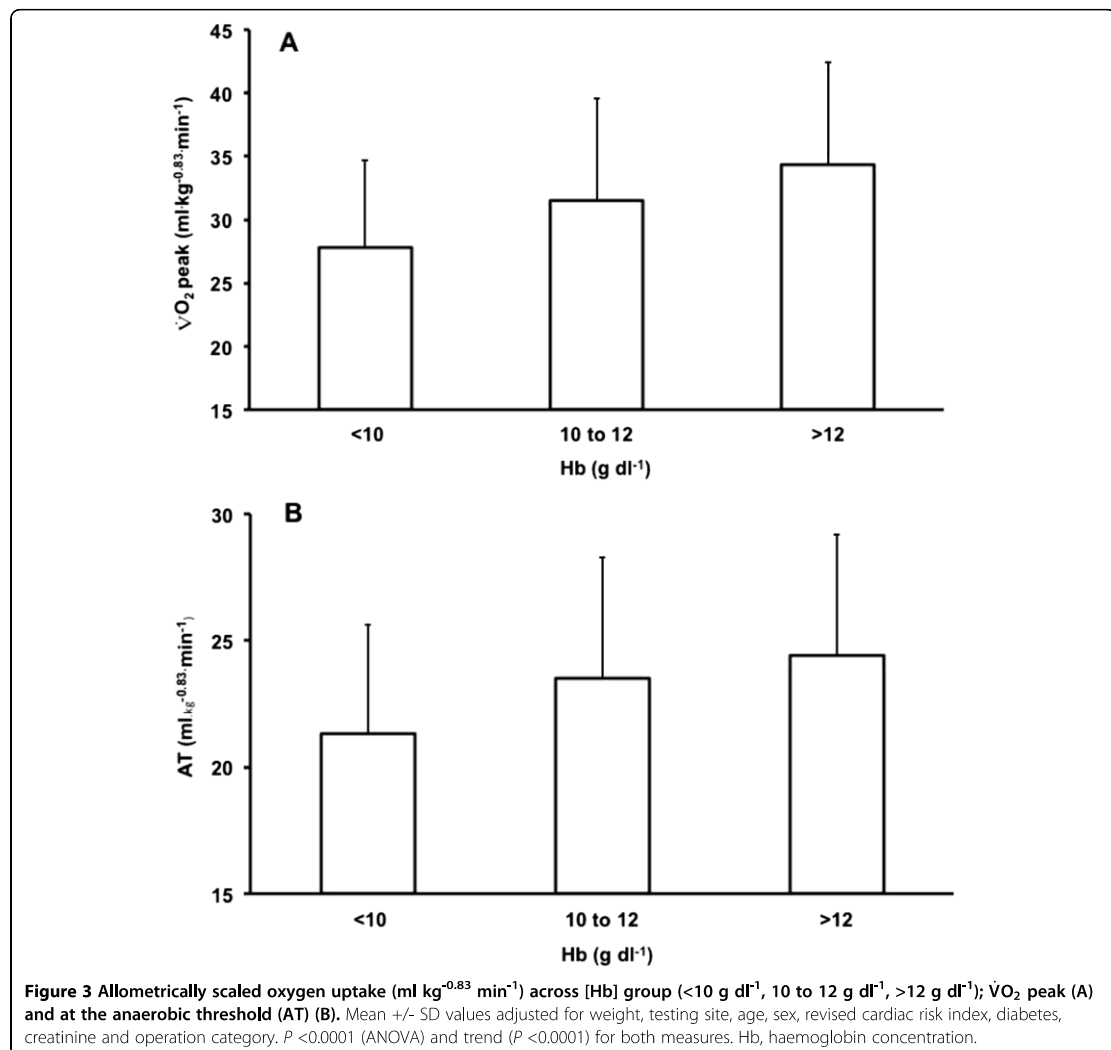
	Haemoglobin concentration			P-value	
	<10 g dl ⁻¹	10 to 12 g dl ⁻¹	>12 g dl ⁻¹	ANOVA	Trend
$\dot{V}O_2$ peak (ml kg ^{-0.83} min ⁻¹)	26.7 (7.8) 83	29.9 (8.4) 352	34.4 (10.1) 1339	<0.0001	<0.0001 ^a
	27.8 (7.1) 83	31.2 (8.2) 352	34.0 (8.7) 1333	<0.0001	<0.0001 ^b
	27.8 (6.9) 64	31.5 (8.1) 291	34.3 (8.1) 1136	<0.0001	<0.0001 ^c
AT (ml kg ^{-0.83} min ⁻¹)	20.9 (4.6) 67	22.7 (5.0) 307	24.4 (5.2) 1257	<0.0001	<0.0001 ^a
	21.3 (4.5) 67	23.2 (4.9) 307	24.3 (5.1) 1251	<0.0001	<0.0001 ^b
	21.3 (4.3) 49	23.5 (4.8) 252	24.4 (4.8) 1068	<0.0001	<0.0001 ^c

$\dot{V}O_2$ peak and AT, mean (SD) number of patients, by haemoglobin classification. AT anaerobic threshold, [Hb] haemoglobin concentration, $\dot{V}O_2$ peak, peak oxygen uptake.

^aAdjusted for weight and testing site.

^bAdjusted for weight, testing site, age, sex.

^cAdjusted for weight, testing site, age, sex, revised cardiac risk index, diabetes, creatinine and operation category.



in our cohort but may independently influence fitness markers in the absence of anaemia. For example, iron deficiency with or without anaemia is associated with reduced fitness [48-50].

This study had some limitations. The observational, cross-sectional and retrospective nature of the data generates causative hypotheses but does not test them [51]. It would have been valuable to assess the association of overall survival and critical care use with both anaemia and oxygen uptake. The [Hb] may be an imprecise measure of blood oxygen carrying capacity, given that its value is influenced by disease- or therapy-related contractions or expansions in plasma volume. For instance, oxygen-carrying capacity may be normal if [Hb] is low

due simply to an increase in intravascular volume. A better measure of oxygen-carrying capacity may thus be total mass of haemoglobin (tHb-mass). The tHb-mass displays a higher correlation with $\dot{V}O_2$ peak ($r^2 = 0.79$) than either blood volume ($r^2 = 0.76$) or [Hb] [52,53]. The relatively small explained variance in AT and $\dot{V}O_2$ peak by [Hb] (oxygen carrying capacity) in the current study suggests that other factors may play an important role in determining aerobic capacity. For example, other physiological factors that may limit $\dot{V}O_2$ peak include pulmonary diffusing capacity, cardiac output and skeletal muscle limitations [54]. Although it is suggested that the AT reflects an imbalance in oxygen demand-supply, that is, that AT reflects onset of anaerobiosis, this is a much-

debated and controversial concept [44,55]. In addition, variations in the AT, to some extent may be explained by inherent discrepancies in its measurement and/or interpretation [44].

Future studies of preoperative cardiopulmonary exercise testing should include [Hb] with long-term survival and quality of life as outcomes as well as considering alternative endpoints measured during exercise testing such as metabolic efficiency and the oxygen pulse [44].

Conclusions

In conclusion, anaemia is common in preoperative patients undergoing elective major surgery. There is an association between haemoglobin concentration and oxygen uptake during exercise, both $\dot{V}O_2$ peak and AT, even after adjusting for measured confounding variables. Future studies may wish to address whether reversing anaemia before surgery improves these values, and thereby increases postoperative survival and function.

Abbreviations

AT: Anaerobic threshold; BMI: Body mass index; CPET: Cardiopulmonary exercise testing; [Hb]: Hemoglobin concentration; RCR: Revised cardiac risk index; tHb-mass: Total haemoglobin mass; $\dot{V}CO_2$: Carbon dioxide; $\dot{V}E/\dot{V}O_2$: Ventilatory equivalent for oxygen; $\dot{V}E/\dot{V}CO_2$: Ventilatory equivalent for carbon dioxide; $\dot{V}O_2$: Oxygen uptake; $\dot{V}O_2$ peak: peak oxygen uptake.

Competing interests

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MPWG leads the Xtreme-Everest hypoxia research consortium and the group have received unrestricted research grant funding from: BOC Medical (Linde Group), Ely-Lilly Critical Care, Smiths Medical, Deltex Medical, London Clinic, Rolex. MPWG runs a number of educational meetings and these meetings have sponsorship from multiple industry partners declared on a meeting-by-meeting basis.
MPWG: Board and Research Council member of the National Institute of Academic Anaesthesia, Co-Chairman of Evidence Based Perioperative Medicine (annual scientific meeting), Co-Chairman of Current Controversies in Anaesthesia and Perioperative Medicine (annual scientific meeting), Co-chairman of National Perioperative CPET Meeting (annual scientific meeting), Co-chairman of KnO2wledge (annual scientific meeting), Member organising group, UK Perioperative Clinical Research Forum (annual scientific meeting), Faculty of Perioperative CPET course, Executive faculty of UK-UlAA Diploma in Mountain Medicine, Editor in Chief of Extreme Physiology and Medicine, Editorial board of Perioperative Medicine and British Journal of Hospital Medicine, Member of the Improving Surgical Outcomes Group. All remaining authors declare that they have no competing interests.

Authors' contributions

JMO, AFO, PJH, CS, JBC, and MS were responsible for data collection, drafting and revising the article and final approval of the version to be published. JAC was responsible for statistical analyses, revising the article and final

approval of the version to be published. MPWG was responsible for substantial contribution to conception and design and facilitating acquisition of data, revising the article and final approval of the version to be published. TR and HEM were responsible for substantial contribution to conception and design, revising the article and final approval of the version to be published. All authors read and approved the final manuscript.

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